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Departamento de Zoología y Antropología Física (Zoología)

FACTORES QUE AFECTAN A LA SUPERVIVENCIA JUVENIL DE LA CIGÜEÑA BLANCA *CICONIA CICONIA*

MEMORIA PARA OPTAR AL GRADO DE DOCTOR
PRESENTADA POR

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Bajo la dirección del doctor
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Ciconia ciconia

TESIS DOCTORAL

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Memoria presentada para optar al título de Doctor en Ciencias Biológicas
por la Universidad Complutense de Madrid
por

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El doctorando **Vº Bº del director de la Tesis**

Fdo.: José Ignacio Aguirre de Miguel **Fdo.: Manuel Fernández-Cruz**

Marzo 2006

A mi familia por mostrarme el camino

A Eva por ayudarme a recorrerlo

CONTENIDOS

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Gracias de todo corazón

INTRODUCCIÓN GENERAL

1.1. MARCO CONCEPTUAL

Estrategias de los reproductores

El éxito reproductor relacionado con la edad ha jugado un papel importante en el campo de las teorías sobre la historia vital (Reid *et al.*, 2003). La diferencia en como éste varía entre individuos jóvenes o viejos ha intentado ser explicada por cuatro bloques de hipótesis diferentes (Wiktander *et al.*, 2001). Estas hipótesis no son excluyentes entre sí se centran en distintos factores y niveles de análisis.

En una primera hipótesis, los *malos* reproductores disminuirán sus oportunidades de supervivencia y de éxito reproductor frente a los *buenos* reproductores (hipótesis de la *mala calidad* (Curio, 1982) e hipótesis de *selección* (Nol & Smith, 1987). La habilidad para sobrevivir puede estar correlacionada positivamente con su éxito reproductor ya que ambos, supervivencia y reproducción, están regulados por las mismas variables, como la condición corporal, la experiencia y la habilidad para interactuar con el medio (Wheelwright & Schultz, 1994). Una segunda hipótesis postula que algunos reproductores no se incorporarán a la población a la edad mínima de reproducción, sino hasta el momento en que estén muy capacitados; es la llamada hipótesis de *reproducción retardada* propuesta por Hamann & Cooke (1986) y posteriormente por Lessels & Krebs (1989). La participación retardada en eventos reproductivos puede producir individuos en mejor condición física en su primer intento reproductivo, y esto a su vez puede dotarles de una ventaja frente a otras aves que se reproducen en etapas más tempranas. Incluso si los individuos demostrasen una mejora entre temporadas sucesivas, las cohortes mas viejas de una población tendrían un éxito reproductor medio más alto debido a la incorporación de individuos con reproducción retardada. Como se puede observar, ambas hipótesis se centran en la proporción variable de fenotipos dentro de una misma población.

Las otras dos hipótesis restantes se centran en la competencia entre individuos. Si un individuo envejece, aumenta su experiencia y esto afecta de manera positiva a su reproducción; es denominada hipótesis de la *fuerza* (Curio, 1982), hipótesis de la *madurez* (Hamann & Cooke, 1986) o *habilidades específicas de la edad* (Wheelwright & Schultz, 1994). Las clases de edad más avanzadas incrementan el porcentaje de buenos reproductores de toda una población. Su éxito aumenta debido a características que cambian con la edad: tamaño mayor, estatus social más elevado o una experiencia más amplia (Wheelwright & Schultz, 1994). En segundo lugar, los individuos deben ajustar su inversión en la reproducción de acuerdo con su probabilidad

de supervivencia o valor reproductivo residual (hipótesis de la *restricción* (Curio, 1982), o del *valor reproductivo residual* (Nol & Smith, 1987) o de la *teoría de la historia vital* (Wheelwright & Schultz, 1994). Los individuos deben incrementar su esfuerzo reproductivo al final de su vida y consecuentemente, aumenta el éxito reproductor de los grupos de edad más avanzada.

Cualquiera de las hipótesis descritas anteriormente pueden actuar simultáneamente dentro de una población. Por tanto, la pregunta que surge en cuanto a la reproducción en función de la edad es: ¿Qué tendencias tienen que ver con los cambios individuales en la experiencia y cuales con los cambios poblacionales por supervivencia?

Se han llevado a cabo estudios a largo plazo para identificar aspectos relacionados con la reproducción, en los cuales los individuos jóvenes son menos exitosos que los adultos (Wooler *et al.*, 1990; Laaksonen *et al.*, 2002; Newton & Rothery, 2002; Saino *et al.*, 2002; Reid *et al.*, 2003). Otros han tratado el éxito reproductor en las últimas etapas de la vida reproductiva en especies longevas (Emslie *et al.*, 1992; Forslund & Larsson, 1992; Rockwell *et al.*, 1993; Part, 2001; Laaksonen *et al.*, 2002; Newton & Rothery, 2002; Nisbet *et al.*, 2002; Anderson & Apanius, 2003; Reid *et al.*, 2003). Estos trabajos a largo plazo presentan mucha dificultad debido a que los individuos de estas especies deben ser datados cuando son jóvenes y pocos estudios han conseguido realizar un seguimiento de estos individuos hasta que se incorporan a los grupos de edad más avanzada. La mortalidad es otro factor que también incide en todos los grupos de edad y por ello solo una proporción de individuos alcanza la fase adulta.

Otros trabajos defienden que el éxito reproductor está fundamentalmente determinado por la capacidad de escapar a la depredación (Newton & Rothery, 2002), un factor de mortalidad que puede ser incidental en muchas especies y, por tanto, relativamente independiente de la edad de los reproductores en cuanto a su tamaño, o condición física, pero no en lo concerniente a su experiencia para evitar a los depredadores (Green, 2001). La contribución a la reproducción de la calidad de los padres frente a eventos accidentales, como la depredación, puede depender o no de la habilidad y experiencia de los padres (Green, 2001; Blanco & Bertellotti, 2002) y puede ser ajustada mediante la exclusión en los análisis de los casos que implican un fallo total en la reproducción, normalmente asociados a episodios de depredación (Newton & Rothery, 2002) o analizando la influencia de la edad en el éxito reproductor y en el tamaño de puesta de manera independiente.

A pesar del interés que presenta el análisis de diferentes parámetros reproductores, para evaluar su influencia en la reproducción según la edad de los individuos, sólo diez estudios han abordado esta cuestión y han medido parámetros relacionados con el éxito reproductor, como el tamaño de puesta y de la pollada, así como elementos relacionados con el fracaso reproductor (Ver tabla 1 en Capítulo 3). Entre los factores analizados, la depredación y la muerte por inanición de los pollos se han revelado como los dos elementos principales que influyen en el éxito reproductor donde la depredación parece ser el factor determinante que influye en el fracaso reproductor en la mayoría de estas especies.

Una de las estrategias adoptadas para la protección frente a depredadores, es el cambio de nidos en la temporada posterior a un episodio de depredación, ya que los depredadores pueden tener memoria a largo plazo para recordarlos. Sin embargo, la fidelidad a los nidos también se considera una estrategia adaptativa en numerosas especies de aves como las ciconiformes (Cezilly *et al.*, 2000). Se ha comprobado que este comportamiento refuerza los lazos de pareja y por tanto la retención de ésta (Cezilly *et al.*, 2000), que puede implicar una mayor tasa de supervivencia para adultos (Bried *et al.*, 2003) y una contribución al mantenimiento de su estatus social (Heg *et al.*, 2003).

La edad y el éxito reproductor en la temporada precedente, son dos factores que afectan la fidelidad al nido (Pyle *et al.*, 2001). Sin embargo en el momento de estudiar ambos factores, es preciso recordar que están correlacionados entre si, ya que los individuos jóvenes tienden a fallar más a menudo en la reproducción que los adultos y presentan, además las tasas más bajas de cambio de nido (Pyle *et al.*, 2001).

La colonialidad también ha sido un factor apuntado como determinante de la capacidad reproductiva en aves (Witenberg & Hunt, 1985), auque puede haber costes asociados a la ésta, como altas tasas de parasitismo y competencia por sustratos de nidificación y materiales (Dubois *et al.*, 1998; Rolland *et al.*, 1998; Szep & Moller, 2000). El éxito reproductor es mayor en colonias grandes que en pequeñas, probablemente debido a que disminuye el índice de depredación (Barbosa *et al.*, 1997)

La edad de los reproductores y la fidelidad a los nidos, son por tanto, dos factores directamente relacionados con la capacidad reproductora de cada individuo y que deben ser tenidos en cuenta al evaluar los factores que afectan a la supervivencia juvenil.

Estrategias de los pollos

Eficacia biológica

La bibliografía existente, asume que la condición física de los pollos al abandonar el nido influye en su probabilidad de supervivencia futura (Weatherhead & Dufour, 2000; Cam *et al.*, 2003; Brown & Roth, 2004). La competencia entre hermanos es una de las fuerzas más importantes para explicar la variación dentro de la pollada en condición física y supervivencia. Los padres, especialmente las madres, pueden alterar esta competencia y por tanto la supervivencia en etapas tan tempranas como durante el desarrollo embrionario, aportando diferentes recursos a distintos huevos dentro de la secuencia de puesta o según su sexo (Slagsvold *et al.*, 1984; Weatherhead, 1985; Bednarz & Hayden, 1991; Gowaty, 1995; Eising *et al.*, 2001; Blanco *et al.*, 2002; Blanco & De La Puente, 2002; Blanco *et al.*, 2003). Además, los padres podrían realizar aportes de alimentación diferenciales en relación con el sexo de sus pollos o el orden dentro de la pollada (Ploger & Medeiros, 2004) o incluso perpetrar infanticidios (Tortosa & Redondo, 1992). En especies con asincronía de puesta, los miembros más mayores de la pollada superan a sus hermanos en cuanto a ingesta de alimento y por ello crecen más rápido (Wiebe & Bortolotti, 1994), aunque los jóvenes pueden compensar estas incidencias adoptando estrategias de crecimiento que les permitan mantener el ritmo respecto a sus hermanos mayores (Nilsson & Svensson, 1996).

En muchas aves, el condición física de los pollos también es un elemento relacionado con la eficacia biológica (Krebs, 1999; Clotfelter *et al.*, 2000). Como el orden de puesta influye en la condición física de los pollos (Wiebe & Bortolotti, 1994), es muy probable que también afecte a la eficacia biológica de los individuos (Cam *et al.*, 2003). Las tasas de mortalidad más altas se producen durante los primeros años de vida (Schmutz & Fyfe, 1987; Roth *et al.*, 2005) y este periodo es particularmente crítico especialmente para especies migratorias cuyo estrés migratorio añade una causa adicional de mortalidad. Los primeros pollos tienen tasas de mortalidad más bajas que el resto de la pollada a lo largo del viaje migratorio debido a su mejor condición física de partida (Cam *et al.*, 2003).

La migración es una estrategia adaptativa que surge para adecuar las demandas de alimentación de los individuos a la disponibilidad temporal de recursos (Pérez-Tris & Tellería, 2002), por tanto y desde un prisma evolutivo, sería de esperar que cambios en la temporalidad del alimento produjesen un cambio en los hábitos migratorios. Este es un fenómeno que parece ocurrir en las poblaciones de cigüeñas de la Península Ibérica (Martínez, 1994; Vergara *et*

al., 2004). Desde los años 80 algunos individuos han sido vistos en noviembre y diciembre en la áreas de cría (Prieto, 2002), cuando tradicionalmente en esta época del año deberían encontrarse en sus zonas de invernada en África. En las últimas décadas, este fenómeno se ha incrementado en muchas regiones españolas donde se han detectado importantes concentraciones invernales (Tortosa *et al.*, 1995; Marchamalo, 2002; Vergara *et al.*, 2004). En la actualidad la invernada en áreas de la Península está muy bien documentada (Marchamalo, 1994; Sanchez *et al.*, 1994). La causa de este cambio en el patrón migratorio ha sido la proliferación en extensión y número de los vertederos de residuos sólidos, lo que supone alimento disponible e ilimitado a lo largo de todo el ciclo anual (Blanco, 1996; Tortosa *et al.*, 2002).

Nos encontramos por tanto ante un interesante experimento natural que permite que, un recurso natural limitante como puede ser el alimento, actualmente esté disponible para toda una población de manera ilimitada, lo que permite observar como este factor puede incidir en variables relacionadas con la eficacia biológica.

Sistema inmunitario

El sistema inmunitario es la defensa más importante con la que cuenta un organismo para defenderse de agentes patógenos. Según Soler (1999) el sistema inmunológico de los vertebrados se articula a tres niveles: procesos de fagocitosis, respuesta mediada por células T, ambos considerados mecanismos de repuesta inespecíficos, y respuesta por medio de anticuerpos, un mecanismo mucho mas específico que produce anticuerpos y linfocitos sensibilizados ante patógenos distintos.

Una medida directa de la respuesta inmune mediada por células T, es la prueba de la fitohematoglutinina. Esta prueba fue aplicada por primera vez en aves de corral (Cheng & Lamont, 1988), pero posteriormente se comenzó a aplicar en aves silvestres (Smits *et al.*, 1999) y actualmente representa un método muy aceptado para evaluar el estado del sistema inmune de los individuos. Diferentes resultados sugieren la importancia evolutiva del sistema inmunitario y sus implicaciones a distintos niveles, tales como la historia vital (Tella *et al.*, 2002), senescencia (Lozano & Lank, 2003), dispersión (Moller *et al.*, 2004), caracteres sexuales secundarios (Gonzalez *et al.*, 1999), tamaño de puesta (Tella *et al.*, 2000), orden de puesta (Roulin *et al.*, 2003), fecha de puesta (Moreno *et al.*, 1998), colonialidad (Tella *et al.*, 2001), coste asociado (Lochmiller & Deerenberg, 2000), disponibilidad de alimento (Hoi-Leitner *et al.*, 2001), decisiones parentales (Johnsen *et al.*, 2000), sexo

(Fargallo *et al.*, 2002) y variaciones interanuales (Jovani *et al.*, 2005).

1.2 OBJETIVOS

El objetivo general de esta memoria es aportar un enfoque novedoso a problemas ya estudiados anteriormente en otras especies y no circunscribirse únicamente a resultados puramente ornitológicos sino extraer conclusiones dentro del campo de la ecología evolutiva.

Se trata por tanto de abordar algunos de los factores que influyen en el desarrollo de los pollos que vienen determinados por las estrategias que adoptan los padres.

Los diferentes capítulos de este estudio podrían agruparse dentro de dos grandes bloques:

1. Diferentes estrategias por parte de los progenitores para optimizar su eficacia reproductora y que indirectamente afectan a los pollos que puedan nacer.

2. Algunos de los efectos que tienen sobre los pollos esas estrategias adoptadas por los padres, mediante diferentes indicadores de la eficacia biológica.

El primer bloque que, aborda las diferentes estrategias de los progenitores respecto a su reproducción incluye dos capítulos. El primero de ellos (***Capítulo 1***), pretende estudiar la influencia de la edad en la reproducción ya que no todos los grupos de edad pueden tener el mismo éxito o producir el mismo número de pollos. La edad puede tener una influencia en el éxito reproductor, circunstancia que puede condicionar tanto el número de pollos que nacen como el éxito o fracaso de una pareja reproductora. Posteriormente, se estudia la fidelidad de estos individuos a su lugar de nidificación (***Capítulo 2***). En este sentido, y en relación con los resultados obtenidos en el capítulo anterior, se analiza cómo el cambio de lugar de nidificación puede estar influido por distintos factores, como la edad de los individuos o su éxito o fracaso reproductor en ese mismo nido en la temporada precedente, y si ésta estrategia reproductora supone algún beneficio desde el punto de vista de la eficacia biológica.

Un segundo bloque, compuesto de dos capítulos, estudia los efectos que tienen sobre los pollos las distintas estrategias adoptadas por los padres. En un primer capitulo se describe cómo el orden en que nacen los pollos puede tener una influencia no sólo en su condición física inmediata sino además en su futura eficacia biológica al incorporarse como reproductores a la población (***Capítulo 3***). Posteriormente se explica cómo las decisiones que adoptan los padres en cuanto al sexo de los pollos y su crecimiento influye en su repuesta inmune celular (***Capítulo 4***).

1.3 METODOS GENERALES

Características físicas y situación

La Cigüeña Blanca (*Ciconia ciconia*) es una ciconiforme sin dimorfismo sexual marcado y con los machos un 5% más grandes que las hembras en tamaño. Se distribuye por todo el Paleartico Occidental desde el oeste de Asia, Asia Menor y Oriente Medio hasta el Norte de África como reproductora (Schulz, 1998). Tradicionalmente se le ha considerado un migrador transahariano. Las cigüeñas que pasan por España corresponden a las de la ruta occidental que discurre por la Península, cruzando por el estrecho de Gibraltar, con zonas de invernada en el Sahel africano entre Senegal y Chad (Bernis, 1959; Diaz *et al.*, 1996; Schulz, 1998). Sin embargo, en las últimas décadas, el número de individuos que pasan el invierno en el sur de la Península Ibérica, ha aumentado considerablemente (Máñez *et al.*, 1994; Marchamalo, 1994; Sánchez *et al.*, 1994; Marchamalo, 2002).

Durante los últimos 20 años, la Cigüeña Blanca en España ha mostrado una considerable recuperación en cuanto a su número de parejas reproductoras, pasando de 14050 en 1948 (Bernis, 1959) a 16643 en 1984 (Schulz, 1999) y 33217 en el último censo nacional de 2004 (Molina & Del Moral, 2005). Este incremento se ha debido a la protección dispensada y a la aparición de nuevos recursos tróficos predecibles y geográficamente extendidos (los Vertederos de Residuos Urbanos), (Donázar, 1992; Blanco, 1996; Medina *et al.*, 1998; Peris, 2003). En la Comunidad de Madrid, el número de parejas reproductoras, ha pasado de 215 en 1984 (Lázaro *et al.*, 1986) a 979-1013 en 2001 (Aguirre & Atienza, 2002) y 1221 en 2004 (Molina & Del Moral, 2005).

Patrón general de emparejamiento

El patrón general de emparejamiento es la monogamia, aunque se han descrito excepcionalmente casos de poliginia (Prieto, 2002). Ponen entre 1 y 6 huevos a mediados de abril (media 3), que incuban durante 32 días. Los pollos están en el nido alrededor de 55 días y tras sus primeros vuelos, se agrupan para formar grandes concentraciones y para iniciar su migración hacia el sur alrededor del mes de julio.

Área de estudio

El trabajo de campo se ha realizado en el período comprendido entre los años 1999 y 2005 en la Comunidad de Madrid. Gran parte de los estudios se han focalizado sobre una colonia que está situada en la zona norte de Madrid entre los términos municipales de Manzanares el Real y Soto del Real (40º44' N, 3º49' W). Dicha colonia se ubica en una dehesa de fresnos (*Fraxinus angustifolia*) situada en una finca privada dedicada a la cría del ganado bovino para carne. La población de esta colonia se ha incrementado

considerablemente en los últimos 16 años pasando de 2-3 nidos en 1989 a 171 en 2005 (obs. pers.), en línea con el incremento ocurrido en el resto de la Comunidad de Madrid. Todos los nidos han sido identificados mediante etiquetas situadas en los árboles y mapeados sobre una foto aérea para poder realizar las labores de seguimiento de los padres, sin necesidad de realizar ningún acercamiento que pudiera alterar la dinámica habitual de la colonia.

Parejas reproductoras

Foto 1. Pareja en nido.

Antes del inicio de la puesta y durante la ocupación de los nidos, se ha realizado un control de los adultos reproductores marcados que ocupan cada nido tanto en la mencionada colonia de Soto del Real, como en otras colonias de la Comunidad de Madrid. Durante los meses de enero, febrero y marzo se han identificado un total de 170 individuos reproductores distintos involucrados en un total de 570 eventos reproductores, es decir, un individuo puede reproducirse en años sucesivos y, cada vez, se considera un evento reproductor diferente.

Puesta y eclosión

Foto 2. Pollos pequeños (15 días aprox.)

Durante la primera quincena de abril se han visitado todos los nidos de la colonia de Soto del Real y se han controlado las fechas de puesta de todos los huevos. Esto ha permitido conocer el orden de puesta de los huevos que han sido marcados mediante un rotulador indeleble y en el orden en que fueron encontrados. También se han tomado medidas del peso, mediante una báscula digital (±0.1 g.), largo y ancho, mediante un calibre digital (±0.01 mm.), de un total de 509 huevos en la eclosión, una muestra representativa toda la colonia.

A pesar de que la bibliografía existente establece 32 días como el periodo de incubación de incubación (Schulz, 1998), en nuestro área de estudio el promedio ha sido 31 días (20-40). Transcurridos 20 días después de la puesta, se visitaron los nidos diariamente para determinar la eclosión de los huevos. y

asignar una posición a cada uno de los pollos nacidos, pintándoles las uñas de distintos colores hasta el momento de su anillamiento. Este se realizó con anilla de metal a los 22 días de edad, momento en que tenían un tamaño suficiente para soportar la anilla. Todos los nidos estudiados han sido asincrónicos, variando la frecuencia de eclosión entre 1.5 y 12 días.

Foto 4. Pollos grandes (35-40 días aprox.)

Volantones

Durante los dos primeros años de recogida de datos, se visitaron un total de 97 nidos con pollos de edad conocida, con el fin de determinar los parámetros adecuados para estimar la edad de los pollos. Así, se tomaron medidas del tarso, pico-cráneo, pico-pluma, ala plegada mediante una regla (±0.1 mm.) y peso mediante una báscula digital (±0.1 g.), resultando el pico-pluma la mejor medida para calcular la edad (Edad = -5.649 + 0.455 pico-pluma (r^2=0.906, p<0.0001).

En una edad comprendida entre los 25 y 50 días, todos los pollos fueron anillados con PVC, pesados, medido su tarso y su pico-pluma. En esta misma visita se tomó una muestra de sangre de la que se extrajo ADN para el sexaje molecular.

En esta visita se procedió igualmente a la evaluación de la respuesta inmune. Se utilizó la prueba de la inyección de fitohematoglutinina (PHA). Se les inyectó a las aves intradérmicamente 0.1 ml. de una solución de 2 mg./ml. de fitohematoglutinina P (Sigma) en una solución de PBS. Se ha usado el protocolo simplificado propuesto por (Smits *et al.*, 1999) que implica una única inyección y omite la inyección de PBS como control en el otro patagio. Las ventajas de eliminar la inyección control es la disminución a la mitad del tiempo de manipulado de las aves, y por lo tanto su estrés, una reducción en la probabilidad de los errores de lectura que implica inyectar en estructuras delgadas como los patagios y una reducción del coeficiente de variación debido a los errores de lectura (Smits *et al.*, 1999). El grosor de cada patagio derecho fue medido usando un calibre digital (±0.01 mm.) en un punto previamente marcado con un rotulador indeleble, antes y 24 horas después de la inyección. La respuesta inmune se ha

estimado como el logaritmo de la diferencia en grosor del patagio entre la media de tres medidas tomadas el día de la inyección y la media de otras tres medidas tomadas al día siguiente. Todas las medidas fueron tomadas por el mismo investigador y siempre con el mismo calibre.

Estadística

En todos los capítulos de esta tesis se ha optado por trabajar con modelos mixtos. Estos modelos permiten emplear al nido y/o al individuo, ya sea pollo o adulto, como unidad muestral evitando los problemas de pseudorreplicación que se derivan de usar los mismos elementos dentro de un mismo análisis. Para ello se incluyen en los modelos el nido, el individuo o el año de cría respectivamente como variables aleatorias según corresponda, lo que permite controlar la varianza debida a las particularidades azarosas y que no pueden ser controladas de cada nido (incluyendo los padres), de cada individuo o las condiciones en diferentes años. Este tipo de análisis tiene además la ventaja de permitir trabajar con variables dependientes que pueden no ajustarse a una distribución normal, o bien con variables en las que sus muestras estén balanceadas. Todos los modelos mixtos fueron realizados con el paquete específico para éstos: SAS (v9.0) con el macro específico para estos modelos GLIMMIX (Littell *et al.*, 1996). El resto de análisis y gráficos fueron realizados con STATISTICA (v6.0).

RESULTADOS Y DISCUSIÓN

Asociación entre edad y reproducción

Se ha analizado la reproducción durante 14 años de 257 individuos involucrados en 793 eventos reproductivos distintos, con el fin de determinar si la edad puede tener un efecto en el éxito reproductor en una especie de larga vida. Los resultados han revelado que los parámetros reproductivos, como el éxito reproductor, no disminuyen a edades avanzadas mientras que el tamaño de pollada disminuye en las hembras que están criando pero no en los machos. La probabilidad de éxito reproductor ha mostrado una tendencia cuadrática para las hembras pero una tendencia lineal positiva para los machos. Esto se resume en que los individuos jóvenes fracasan en la cría más a menudo que los adultos, pero cuando tienen éxito consiguen sacar más pollos que los individuos más viejos. Por tanto, el fracaso en la reproducción juega un papel importante sobre todo en los primeros años de la vida reproductiva de los individuos.

Los padres juegan un papel importante en el éxito reproductor durante la temporada de cría. En muchas especies, la depredación es un elemento determinante en el éxito reproductivo. Sin embargo, en especies coloniales y de gran tamaño, como la Cigüeña Blanca, la influencia de este factor es más relativa. En la población objeto de estudio en

esta tesis, solo el 4% de los nidos han sido depredados. La causa más importante de muerte de los pollos es la inanición. Por ello esta muerte casi siempre suele suceder a los pollos más jóvenes dentro de cada pollada, ya que están en clara desventaja respecto a sus hermanos mayores en cuanto a la recepción de alimento (Zielinski, 2002). La experiencia de los padres en la búsqueda del alimento, determina su capacidad para sacar adelante más o menos pollos. Esta experiencia se va adquiriendo con los años (Forslund & Pärt, 1995) y los individuos más mayores tienen acceso a recursos de más calidad, por su situación de dominancia, adecuación de los momentos a los recursos disponibles y conocimiento previo de las fuentes de alimentación (Pärt, 2001).

Dos mecanismos parecen estar involucrados en el éxito reproductor. En primer lugar, el aprendizaje que aumenta la capacidad en actividades como la búsqueda de alimento, la situación social, etc, dando como resultado un cuidado parental de más calidad y un acceso más fácil a los recursos (Rutz *et al.*, 2006). En segundo lugar, el nivel óptimo de esfuerzo parental que puede incrementarse con la edad debido a la reducción del valor residual reproductivo (Hamann & Cooke, 1986). Es decir, cuantos menos años le quedan al individuo para reproducirse, más energía puede invertir en la reproducción presente sin preocuparse demasiado en si podrá reproducirse o no en el futuro (Wiktander *et al.*, 2001).

También se han encontrado diferencias respecto al sexo en los parámetros reproductores relacionados con la edad, lo que podría explicarse si se confirma que los machos entran en la población reproductora a una edad más temprana que las hembras y por tanto alcanzan su madurez reproductiva antes, tal y como apuntan recientes estudios sobre esta especie (Prieto, 2002). En un futuro, sería interesante realizar estudios a largo plazo, con ambos miembros de la pareja marcados, para determinar en qué momento el macho entra en la población reproductora, con qué hembras se empareja y hasta qué momento lo hace.

Fidelidad a los nidos

La fidelidad al nido es una variable que produce beneficios en términos globales dentro de la historia vital de un individuo. En algunas especies, el éxito o fracaso, el número de pollos producidos en temporadas anteriores y la edad, están correlacionados con la fidelidad a los nidos (Hoover, 2003).

Este parece ser el caso de la Cigüeña Blanca, ya que los resultados de este trabajo muestran que los reproductores más jóvenes mostraron fidelidades al nido menores que los adultos, corroborando estadísticamente las afirmaciones de (Schulz, 1998). Los individuos con una menor tasa de fracaso reproductivo y mayor número de pollos

volados, son además los que presentan una menor tasa de cambio de nido. Los machos más productivos tienden a cambiar con una frecuencia menor de nido. Por lo tanto, la edad y la productividad están claramente correlacionadas.

En general, estas aves tienden a cambiar menos de nido cuanto más mayores son, lo que indica que la experiencia también juega un papel clave en este comportamiento. Los individuos jóvenes de especies coloniales ocupan los nidos marginales y esto, unido a su inexperiencia, probablemente hace que tengan un éxito reproductor menor que los adultos. La experiencia también les permite defender su nido de manera más eficiente frente a posibles competidores (Prieto, 2002). Los individuos de mediana edad, son capaces de sacar adelante sus pollos y tienen en ese punto la experiencia suficiente para mantener su nido durante temporadas de crías sucesivas.

Los dos factores que determinan más claramente la fidelidad a los nidos, en esta especie, son la edad y la productividad en la temporada precedente. Los individuos más viejos tienen una tasa de fidelidad mayor, a pesar de presentar productividades menores que los jóvenes. La interacción significativa entre la edad y el sexo en relación con la productividad, podría indicar que los individuos de cada sexo, entran en la población reproductora a distintas edades como ya han apuntado algunos autores como Prieto (2002), y este comportamiento afecta su tasa de fidelidad a los nidos. Los jóvenes generalmente llegan a las zonas de cría después que los adultos (Barbraud & Barbraud, 1999; Barbraud *et al.*, 1999), viéndose obligados a elegir entre los nidos disponibles y sin posibilidad de defender sus antiguos nidos frente a cualquier adulto que los haya ocupado. La interacción significativa entre el sexo y la productividad en el año anterior en relación con la tasa de fidelidad a los nidos debe ser abordada en profundidad en futuros estudios.

Los resultados también muestran que los individuos que cambian de nido en temporadas sucesivas tienen un mayor fracaso reproductor, aunque no se ha encontrado una diferencia en el número de pollos producidos entre parejas que cambian o que se mantienen en el mismo nido. Este resultado sugiere lo costoso que puede ser energéticamente el cambio de nido. En las cigüeñas, los cambios de nido suelen estar asociados a divorcios (Schulz, 1998). El cambio de pareja en aves longevas implica una ineficiencia reproductiva inicial hasta que los dos miembros de la pareja se adaptan uno al otro (Heg *et al.*, 2003). En esta línea de estudio, la experiencia previa con la misma pareja parece mejorar el éxito reproductivo (Pyle *et al.*, 2001). En esta tesis se muestra como este coste puede implicar un éxito reproductor menor en aquellos

individuos que cambian de nido. En el futuro, las líneas de investigación en este aspecto deberán estar encaminadas hacia: identificar los grados de fidelidad al nido y a la pareja, los costes energéticos que pueden suponer los casos de divorcio y la fidelidad y determinar si estos costes son iguales para ambos sexos. La mayor parte de los estudios realizados hasta la fecha separan un sexo de otro por la dificultad que implica identificar parejas en las que los dos miembros estén marcados (Espie *et al.*, 2004). Se podría también identificar la verdadera función de estos cambios de nido, mas allá de sus causas. Para ello habría que caracterizar la bondad de los nidos y determinar las causas finales de estos cambios.

En conclusión, los resultados indican que la fidelidad al nido representa una estrategia adaptativa para incrementar la eficacia biológica de esta especie.

Eficacia biológica

Como ocurre en otras especies, en su etapa inicial de desarrollo en el nido, las cigüeñas blancas, no presentan diferencias en la masa corporal en relación a su orden de eclosión. Estas diferencias empiezan a ser latentes a partir de la segunda semana de vida, en la que los pollos más mayores empiezan a tener un tamaño mayor que sus hermanos, lo que les da una ventaja adaptativa sobre los pequeños. Se ha demostrado en varias especies que la masa corporal en el momento del vuelo, está relacionada con la supervivencia futura (Krebs, 1999; Clotfelter *et al.*, 2000) y por tanto este parámetro estará relacionado con su eficacia biológica futura.

Los resultados indican que uno de los primeros pasos para determinar la eficacia biológica de los individuos es su orden de eclosión dentro de la pollada. Se ha encontrado que los primeros pollos nacidos en cada pollada tienen un éxito reproductor y un número de pollos volados menor en sus primeros años de vida que sus hermanos pequeños. Esta es la primera evidencia de que pollos en peor condición se reproducen mejor en sus primeros años de vida reproductiva. Sin embargo, es posible que para los primeros pollos eclosionados, esta aparente desventaja en sus primeros años de vida sea compensada después, a lo largo de la vida reproductiva completa de estas aves. Es necesario un estudio a largo plazo de la historia vital completa de estos individuos, con el fin de confirmar estos novedosos resultados.

Una explicación posible para esta diferencia en los parámetros reproductivos relacionados con el orden de eclosión puede estar en las diferentes estrategias migratorias que adoptan los individuos. Las estrategias migratorias están limitadas por la condición física que tiene un individuo al abandonar el nido, momento en el cual cesan los cuidados

parentales. El viaje migratorio representa uno de los mayores costes energéticos que debe soportar un ave (Schaub & Jenni, 2001) y los últimos pollos nacidos tienen una probabilidad más alta de fracasar debido a su masa corporal inferior (Michard *et al.*, 1997).

El crecimiento de los basureros representa una fuente de alimento disponible todo el año, especialmente durante el invierno, momento en el que se reducen drásticamente los recursos tróficos naturales. Los pollos más pequeños pueden beneficiarse de su desventaja *a priori*, disponiendo de una fuente de alimento constante. Podría entonces darse una *contra-selección* evolutiva que hace que los pollos que parten en desventaja en el nido, sean los más aptos en términos de éxito reproductor, en sus primeros años. Este aspecto indica la plasticidad de la selección frente a nuevos y recientes recursos impuestos por el hombre y la capacidad adaptativa a corto plazo de determinadas poblaciones.

No queda demasiado claro, a la luz de los datos disponibles, por qué los primeros pollos migran si disponen de recursos suficientes en su lugar de nacimiento. Sin embargo, se puede apuntar la posibilidad de que en realidad son los pollos pequeños los que no pueden migrar o tienen que realizar un viaje más corto, por lo que aparecen antes en los lugares de cría. Sin embargo se deben abordar estudios encaminados a evaluar la ventaja que los primeros pollos pueden obtener de un viaje migratorio más largo.

La vuelta más adelantada de individuos nacidos más tarde resulta en un inicio de la reproducción más temprano, lo que favorece su éxito reproductor y puede minimizar el tiempo dedicado a buscar y ocupar un nuevo lugar (Tryjanowski *et al.*, 2004) y por tanto permitir aumentar el tiempo dedicado a acumular recursos y obtener una mejor condición de partida antes de la reproducción.

Los resultados muestran, por tanto, que el orden de eclosión es un factor relacionado con la eficacia biológica y las decisiones individuales tras el abandono del nido van a condicionar la vida reproductiva de los individuos.

Respuesta inmune

Este aspecto de la estrategia vital de los individuos también puede estar influido por sus padres. Se acepta que existe un equilibrio entre la repuesta inmune y el crecimiento (Lochmiller & Deerenberg, 2000; Soler *et al.*, 2003); por lo tanto los individuos de mayor tamaño serán más costosos de producir para sus padres (Torres & Drummond, 1999). Los padres pueden distribuir los recursos para manipular la competencia entre hermanos (Magrath *et al.*, 2003), lo que puede ser suficiente para desequilibrar la balanza y sacrificar la capacidad de inmunocompetencia

para obtener tamaños más grandes a nivel embrionario. Los resultados demuestran que un aumento del peso del huevo y por consiguiente de los recursos disponibles para el individuo, puede ser responsable de una peor respuesta inmune.

Después del nivel embrionario, en especies con un ligero dimorfismo sexual en talla, la capacidad inmune de los machos está generalmente menos desarrollada que la de las hembras. Estos efectos están causados por el efecto inmunosupresor de la testosterona (Folstad & Karter, 1992; Moreno *et al.*, 2001): los resultados también reflejan esta tendencia debido a que los machos presentan peor respuesta inmune. Por lo tanto, se puede pensar que el efecto inhibidor de la respuesta inmune de la testosterona tenga un mayor peso específico en el efecto que el equilibrio entre el crecimiento y el desarrollo de la inmunidad anteriormente mencionado. Estas diferencias sexuales pueden ser las causantes de una más alta prevalencia de infecciones y puede tener consecuencias ecológicas y biológicas como por ejemplo una entrada en la senescencia o una vida reproductiva más corta (ver Capitulo 1 y Lochmiller & Deerenberg, 2000).

La nutrición también tiene un efecto sobre la respuesta inmune, en línea con el equilibrio anteriormente mencionado (Hoi-Leitner *et al.*, 2001). A mayor cantidad de recursos, mayor probabilidad de distribuirlos en mayor medida entre las dos opciones. En la cigüeña, esta distribución de recursos generalmente favorece a los pollos mayores (Ploger & Medeiros, 2004). Los resultados están en línea con otros existentes (Muller *et al.*, 2003) sobre una menor respuesta inmune de los últimos pollos nacidos. También la composición de la yema de la que proceden esos individuos puede ser importante, ya que se ha visto que las concentraciones de testosterona aumentan en las últimos huevos (Sockman & Schwabl, 2000) y hay que recordar el efecto inhibidor que esta hormona ejerce sobre el desarrollo del sistema inmune.

Se ha propuesto una variabilidad temporal en la respuesta inmune y algunos autores sugieren la necesidad de introducir la variable temporal en estudios sobre respuesta inmune. A la vista de nuestros resultados, esta variabilidad temporal puede ser inducida por la variabilidad en los recursos ambientales, especialmente el alimento, fluctuante en la zona de estudio (Jovani *et al.*, 2005), pero en el caso de la colonia estudiada y gracias a la presencia de los vertederos de residuos sólidos, esta disponibilidad de alimento es constante. En futuros estudios deberían evaluarse los mismos parámetros que han influido en la respuesta inmune y determinar si han variado o no entre años.

CONCLUSIONES

- La edad de los padres tiene una influencia directa sobre su éxito reproductor. La experiencia puede ser una de las causas que explique las diferencias en el éxito reproductor entre individuos viejos y jóvenes.

- La fidelidad al nido puede ser considerada como una estrategia adaptativa ya que puede aumentar la eficacia biológica de los individuos. La edad y el éxito reproductor en el año precedente determinan la fidelidad a los nidos en la temporada de cría.

- El orden de eclosión determina la eficacia biológica de un individuo en sus primeros años de vida reproductiva. Los primeros eclosionados son los que peor éxito reproductivo obtienen en estos primeros años. Es probable que el aporte de alimento constante a lo largo del año que suponen los basureros, compense la peor condición física en el momento de abandonar el nido de los últimos pollos eclosionados, permitiéndoles realizar un viaje migratorio más corto y llegar antes a las zonas de cría que sus hermanos mayores con el consiguiente beneficio reproductivo.

- La respuesta inmune en pollos de cigüeña esta mediada por su posición en la pollada, su sexo y el peso que tenía el huevo del que proceden. Existe, por tanto, un equilibrio entre el crecimiento corporal y el desarrollo del sistema inmune y Es posible que la producción de determinadas hormonas implicadas en la expresión de caracteres sexuales secundarios (p.ej. testosterona) tengan un efecto inhibidor sobre la respuesta inmune..

- Los padres juegan un papel fundamental en las expectativas de vida y la eficacia biológica de su progenie, ya sea en forma directa como productores de más o menos pollos y de mayor o menor calidad, como indirectamente, favoreciendo determinadas posiciones en la pollada o sexos. Sin embargo, las decisiones individuales adoptadas por los pollos, como la estrategia migratoria o la fidelidad al nido, también determinan en parte su eficacia biológica futura.

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1 La influencia de la edad en la reproducción de las Cigüeñas Blancas: los jóvenes fuertes y torpes se transforman en expertos cansados en la vejez

Este capítulo reproduce el texto íntegro del siguiente manuscrito:

Aguirre, J.I. & Blanco, G. (*En revisión*). The influence of age on breeding performance in white storks: inept strong young breeders turn into tired experts at old age. *Journal of Avian Biology.*

RESUMEN

En este capítulo se han analizado los parámetros reproductores de individuos de Cigüeña Blanca *Ciconia ciconia* marcadas con PVC que han sido estudiados en la Comunidad de Madrid (centro de España) desde 1980 a 2004. El objetivo ha sido mostrar alguna evidencia de senescencia en el curso de un estudio a largo plazo. Se ha utilizado una muestra de individuos marcados de edad y sexo conocido para tomar tres medidas de los parámetros reproductores: *éxito reproductor* (definido como el número de pollos volados en cada intento de cría incluyendo los ceros), *tamaño de puesta* (definido como el número de pollos volados en cada intento de cría sin incluir los ceros) y *probabilidad de fracaso en la reproducción* (una variable binaria definida por si hubo (1) o no hubo (0) pollos volados en cada intento de cría). Los datos han revelado que el éxito reproductor no decreció a edades avanzadas mientras que el tamaño de puesta decreció para hembras pero no para machos. La probabilidad de fracaso en la reproducción muestra una tendencia cuadrática para hembras pero una tendencia lineal para machos. Esto indica que el fracaso reproductor es importante durante los años iniciales y centrales de la vida de un individuo pero no tan importante al final de su vida reproductora. Las cigüeñas jóvenes fallan más a menudo aunque suelen sacar adelante más pollos cuando lo hacen que las cigüeñas mas viejas, que sacan menos pero con una probabilidad de éxito mayor.

The influence of age on breeding performance in white storks: inept strong young breeders turn into tired experts at old age

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ABSTRACT

The breeding performance of individually PVC-ringed White Storks Ciconia ciconia was studied in the Madrid province (Central Spain), from 1980 to 2004. Our objective was to show some evidence of senescence on reproductive success in the course of a long-term study. A sample of known age and sex individuals was used for analysing three measures of reproductive performance namely breeding success, brood size and the probability of breeding failure. The data revealed that breeding success did not decline at old ages while brood size declined for females but not for males. The probability of breeding failure showed a quadratic tendency for females but a linear positive tendency for males. These results then show that young white storks fail more often, though if they succeed, they raise more nestlings. On the other hand, old storks raise lower amounts of offspring but whit a very high success probability. We suggest that total nest failure is important during the first and middle years of life but less important at the end of life history.

INTRODUCTION

Reproduction and survival rates related to age have determined an important field of life history theory (Reid *et al.,* 2003). Several hypotheses have been proposed to explain low breeding success at young age classes and improvement at old age classes (see Wiktander *et al.,* 2001 for review). Two of these hypotheses refer to individual competence. Older individuals tend to be better at foraging and nest defence against predator activities (Curio, 1983; Hamman & Cooke, 1987; Wheelwright & Schultz, 1994) and breeding investment increases with age compromising remaining residual reproductive value, so breeding success in the latter years of life may increase (Curio, 1983; Nol & Smith, 1987; Wheelwright & Schultz, 1994). Reproductive life of an individual compromises the equilibrium between these two tendencies since they are not mutually exclusive.

Most studies on age-specific reproductive performance in birds have focused on changes in the number of eggs laid and offspring fledged (Emslie *et al.,* 1992; Pärt *et al.,* 1992; Wiebe & Martin, 1996; Nisbet *et al.,* 2002; Saino *et al.,* 2002; Laaksonen *et al*., 2002; Anderson & Apanius, 2003). Less attention has been paid to the causes reducing or increasing these parameters as well as to the influence of total breeding failure (e.g. by predation) on breeding performance according to age at population and individual levels. Breeding success (or failure) has been argued to be mainly determined by the ability to escape predation (Newton & Rothery, 2002), a mortality factor that may be incidental in many species and, therefore, relatively independent of the quality of breeders in terms of size, vigour and health but not of their experience and ability to deter predators (Green, 2001). Few bird species are relatively free of nest predators. Therefore, breeding failure is often related to predation (Newton & Rothery, 2002). Distinguishing whether age-specific reproductive performance depends on the quality of individuals to rear more offspring or to deter predation may be confounded by the difficulty to determine the causes of total breeding failure and the extent of partial predation of clutches/broods. The contribution of individual quality versus incidental events such as predation, which may depend or not on the ability and experience of parents (Blanco & Bertellotti, 2002; Green, 2001), may be assessed more readily by excluding cases of total breeding failure (zero values) from the analyses (e.g. Newton & Rothery, 2002) or by analysing the influence of age on breeding success and brood size separately.

Despite the potential interest of analysing different breeding parameters to assess their influence in explaining patterns of age-specific breeding performance, we found only ten published studies that separately assessed

measures of breeding success such as clutch or brood size as well as measures of breeding failure according to breeders age (Table 1). Such studies were conducted on a variety of species with contrasting breeding strategies and potentially variable factors causing clutch/brood reduction and total breeding failure, which may influence patterns of age-related breeding performance differently. Among these factors, predation and starvation arise as the main factors influencing variation in breeding success, of which predation seems the only or most important factor influencing the probability of total breeding failure in most studied species (Table 1).

We tested the effect of age on breeding failure, reproductive success, and the probability of breeding failure. Our prediction is that reproductive success of the breeding males and females vary with age. We focused on the incidence of senescence on the population-level reproductive performance and discuss the implications of senescence in the reproductive strategies of storks. We first assessed the causes of breeding failure and then assessed whether senescence is characterized by experience to avoid total breeding failure.

METHODS

Individual banding and resighting

The study was conducted in the Madrid province (7740 km^2), central Spain. In this area an increase in population numbers of white storks has occurred from 1984 (215 breeding pairs, Lázaro *et al*. 1986) to 2001 (979-1013 pairs, Aguirre & Atienza 2002) and 2004 (1220 pairs, Aguirre, unpublished data).

From 1980 to 2004, 3844 nestlings were banded in their nests with different kinds of numbered metal and PVC bands, at an age of 40 to 50 days. Out of these, 349 chicks were banded with a small standard numbered metal band, 144 with a large metal ring and 3351 with PVC bands with an alphanumeric code. The latter two manners of banding allowed reading the numeric codes from a distance up to 400 m away using telescopes.

From 1989 to 2004 we searched for banded breeding birds within the province of Madrid and identified them by reading their band codes by telescopes when perched on their nests. Overall, a total of 793 breeding events where at least one adult was marked were considered comprising 275 different individuals (89 males, 81 females, 105 undetermined).

Species	Trait	Trend	Main cause of nest mortality	Reference
Red-billed choughs *Pyrrhocorax pyrrhocorax*	F. Y. B. S.	A^p A^p	predation, starvation Predation	Reid *et al* 2003
Common Goldeneye *Bucephala clangula*	F. Y. B. S.	A +		Milinoff *et al* 2002
Sparrowhawk *Accipiter nisus*	F. Y. B. S.	V^p +	predation, starvation predation	Newton & Rothery 2002
Brown Thornbill *Acanthiza pusilla*	F. Y. B. S.	+ +	predation, starvation Predation	Green 2001
Lapwing *Vanellus vanellus*	F. Y. B. S.	+ 0	Starvation	Parish *et al* 2001
Tree Swallow *Tachycineta bicolor*	F. Y. B. S.	A A	Predation, starvation Predation	Robertson & Rendell 2001
Seychelles warbler *Acrocephalus sechellensis*	F. Y. B. S.	A A	Unattended Unattended, starvation	Komdeur 1996
Lesser snow-goose *Chen caerulescens caerulescens*	F. Y. B. S.	A A	Predation, starvation Parent experience	Rockwell et al 1993
Barnacle goose *Branta leucopsis*	F. Y. B. S.	A V	Starvation Parent experience	Forslund & Larsson 1992
White Stork *Ciconia ciconia*	F. Y. B. S.	A +	Starvation Abandonment	This study

Table 1. Studies that have tested tendencies in fledged young and breeding success at different age of individuals. Trend represents tendencies either u-shape that is low in early stages better in middle life and low again during late stages (A); high in the early stages low in middle life and high again during the late stages (V), increase with age (+), decrease with age (-) and no significant trend with age (0). If a super index (p) is represented independent and dependent variables were tested before and beyond empirical peaks in performance. Traits have been reduced to fledged young (F.Y.) and breeding success (B.S.) although most studies have included many other reproductive traits.

Age was determined by identification of band codes of individuals marked as nestlings. We excluded from the analysis all the adults that were not ringed as nestlings. Many of the marked adults were identified during several years in the same or different nests, although not always in consecutive years. A total number of 699 breeding events where at least one adult was marked and bred for more than one year were recorded. Among them, 182 different individuals were involved in these

events (63 males, 55 females, and 64 undetermined).

Sexing procedures

Sex of breeding individuals was determined by observation of copulatory behaviour at the nest (Cramp, 1977; Schulz, 1998). In addition, many nestlings were sexed by molecular procedures (Fridolfsson & Ellegren, 1999) using DNA extracted from blood obtained by brachial venipuncture during banding at an age of 40-50 days. Many of these nestlings were found subsequently breeding.

We made several visits to the nests with banded breeders to confirm breeding and to obtain data about the output of breeding. Breeding was confirmed if egg laying occurred, which was assessed by recording incubation behaviour of banded birds. Afterwards, the number of fledglings was recorded when nestlings were big enough to be seen over the top of the nest but before they could fly.

Breeding failure

To determine the incidence of several factors involved in breeding failure, an average of 4.5 visits per week were conducted to a single colony (Prado Herrero) over a three breeding season period, with an average of 138 nests holding breeding pairs laying eggs each year. All the nests (placed above Ash *Fraxinus angustifolius*) were checked using a mirror attached to a pole to control laying, hatching and fledging periods. Whenever anomalies were observed we used a ladder to access the nest and confirm dead or disappearance of any of the eggs of chicks aimed to assess the potential cause of failure during the first two years of study.

Statistical Analyses

We analysed the data set with a generalized linear mixed model (GLMM) to overcome the problems of pseudoreplications by using the GLIMMIX macro of SAS (Littell *et al.,* 1996) to test for relationships between age of breeding white storks and breeding performance. We considered three main parameters of breeding performance, that is, (1) *breeding success*, defined as the number of offspring fledged per breeding attempt (including zeros from total breeding failure), (2) *brood size*, defined as the number of offspring fledged during successful breeding attempts (excluding zeros) and (3) the *probability of breeding failure*, a binary variable defined by whether (1) or not (0) any offspring fledged from each breeding attempt. Given that only a small number of the banded individuals were both members of the same

Sex	Trait	Variables	Parameter estimate (*SE*)	F	*d.f.*	P
Females	**Breeding success**	Age	0.3316 (0.06053)	30.01	1,122	<0.0001
		Age^2	-0.01724 (0.003684)	21.90	1,122	<0.0001
		Linear trend before peak (0-4)	0.4811 (0.1444)	11.10	1,26	0.0026
		Linear trend after peak (5-17)	-0.02385 (0.01734)	1.68	1,88	0.1746
Males	**Breeding success**	Age	0.3587 (0.08231)	19.00	1,128	<0.0001
		Age^2	-0.02352 (0.006128)	14.72	1,128	0.0002
		Linear trend before peak (0-4)	0.3774 (0.09590)	15.48	1,44	0.0003
		Linear trend after peak (5-17)	-0.02646 (0.02850)	0.86	1,50	0.3577
Total*	**Breeding success**	Age	0.2549 (0.04075)	39.12	1,307	<0.0001
		Age^2	-0.01387 (0.002681)	26.78	1,307	<0.0001
		Linear trend before peak (0-4)	0.3733 (0.7019)	28.28	1,93	<0.0001
		Linear trend after peak (5-17)	-0.01410 (0.01410)	1.00	1,151	0.3189
Females	**Brood size**	Age	0.0787 (0.03809)	4.29	1,76	0.0418
		Age^2	-0.00430 (0.002243)	3.67	1,76	0.0593
		Linear trend before peak (0-6)	0.04788 (0.03744)	1.64	1,15	0.2204
		Linear trend after peak (7-17)	-0.03248 (0.01725)	3.54	1,36	0.0678
Males	**Brood size**	Age	0.1432 (0.04634)	9.55	1,79	0.0028
		Age^2	-0.01080 (0.003357)	10.34	1,79	0.0019
		Linear trend before peak (0-6)	0.08029 (0.02529)	10.08	1,43	0.0028
		Linear trend after peak (7-17)	-0.05118 (0.0355)	2.08	1,17	0.1676
Total*	**Brood size**	Age	0.06647 (0.02522)	6.94	1,201	0.0091
		Age^2	-0.00409 (0.001618)	6.39	1,201	0.0123
		Linear trend before peak (0-6)	0.04429 (0.01882)	5.54	1,89	0.0208
		Linear trend after peak (7-17)	-0.03044 (0.01532)	3.95	1,67	0.0510

Table 2. GLMM with Poisson error and log link function on breeding success (including zeros) and brood size (excluding zeros) in breeding white storks. Individual identity and year have been considered as random factors x (see text). * includes sexed and non-sexed individuals.

breeding pair (n = 19 pairs), analyses were conducted for males and females separately. In addition, we pooled all data, including non-sexed individuals, in order to maximize sample sizes.

We also conducted analyses at the population level. They included all data across individual birds within years independently of the number of years that particular individuals were recorded as breeders. To allow for the potential pseudoreplication from including different breeding attempts of the same birds at different ages and years we included individual bird identity and year as random effects. For the analysis of breeding success and brood size (response variables), we performed GLMMs with Poisson error and log link where age was the explanatory variable. We included the squared term of age to assess the quadratic pattern typically observed in breeding performance in relation to age in birds (Forslund & Pärt, 1995). However, significant quadratic relationships did not demonstrate a significant increase or decrease of the response variable before or beyond the peak of breeding performance at an intermediate age. Therefore, we also conducted separate analyses for breeding birds at ages younger and older than that peaking in breeding success and brood size namely, 4 for breeding success and 6 for brood size. For the analysis of the associations between the probability of breeding failure and age at the population level we conducted GLMMs with binomial error and logarithmic link where breeding success (at least one nestling fledged) was considered as 1 and breeding failure (no fledgling) as 0. We also performed separate analyses for younger and older birds than 9 years old, the peak in the probability of breeding success. We considered individual identity and year as random factors in the manner of a randomised complete block design to avoid pseudoreplication (Hurlbert, 1984). All tests are two-tailed.

RESULTS

Causes of breeding failure

A total of 1563 eggs from 325 nests were controlled from laying date to hatching. Only 74% of the eggs hatched. No predation episodes on eggs were recorded but we must take into account that this species eliminates very soon any residual of the eggshell from the surface of the nest. Although literature assumes siblicide and infanticide (Tortosa & Redondo, 1992; Redondo *et al.,* 1995; Zielinski, 2002) as the possible cause of death of nestlings at younger age, we have not been able to precisely determine this point due to the fact that observations were not done over a long period of time in order to avoid disturbance. We recorded very rarely the loss of the entire brood, which suggesting that no predation took place against any particular nest. Most of the dead nestlings were affected

Sex	Trait	Variables	Parameter estimate (*SE*)	F	*d.f.*	P
Females	**Failure/Success**	Age	0.8581 (0.1896)	20.48	1,122	<0.0001
		Age^2	-0.04334 (0.01130)	14.72	1,122	0.0002
		Linear trend before peak (0-9)	0.4113 (0.1035)	15.79	1,77	0.0002
		Linear trend after peak (10-17)	-0.2555 (0.1417)	3.25	1,18	0.0880
Males	**Failure/Success**	Age	0.6569 (0.2420)	7.37	1,129	0.0076
		Age^2	-0.03397 (0.01888)	3.24	1,129	0.0744
		Linear trend before peak (0-9)	0.3358 (0.0954)	12.61	1,111	0.0006
		Linear trend after peak (10-17)	11.6457 (3.7507)	9.64	1,4	0.0360
Total*	**Failure/Success**	Age	0.6409 (0.1219)	27.65	1,308	<0.0001
		Age^2	-0.03239 (0.007938)	16.65	1,308	<0.0001
		Linear trend before peak (0-9)	0.2802 (0.05901)	22.55	1,240	<0.0001
		Linear trend after peak (10-17)	-0.1381 (0.2124)	0.42	1,31	0.5204

Table 3. GLMM with binomial error and logistic link function breeding on failure (0) or success (1) in white storks. Parameter estimates and SE for the levels of fixed factors were calculated considering breeding success as 1 and failure as 0. Individual identity and year have been considered as random factors (see text). Total* includes sexed and non-sexed individuals.

by starvation based on the lower weight and worse physical condition of the nestlings dying first (author's unpublished data). A 25% of the hatched chicks died in the first weeks of life. Mortality peaks of small nestlings are related with periods of rain or cold weather (Jovani & Tella, 2004; authors unpublished data). We found that only 4% of the nestlings that were not able to fledge were predated based on rests of nestlings found in the nest or surroundings and partially consumed by carnivorous. An additional 2% of the fledglings died because they fell down from the nest.

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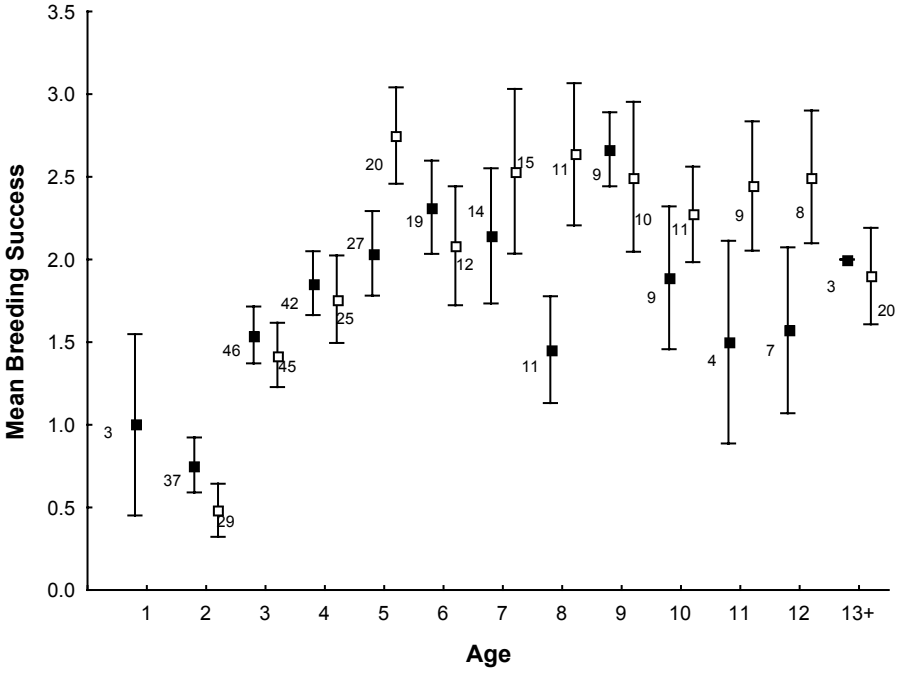

Figure 1. The distribution of brood size with respect to age for white stork in the Province of Madrid. Total includes males (solid square), females (shaded square), and non-sexed individuals. Both nests with fledged young and failed nests are represented. All age classes include known age individuals. Age-class 13+ is a composite of individuals 13 to 17 years old. Sample sizes are indicated.

Associations between age and breeding performance

There was a significant effect of age^2 on breeding success (Figure 1) (including individuals that failed in the reproduction) and brood size (Figure 2) (only individuals that succeed), both for females and males (Table 2). Given that both analyses showed similar results, we performed a third analysis including non-sexed individuals in order to increase the sample size; this analysis showed similar results to that from the analyses for males and females separately (Table 2). These results indicate that there was a quadratic relationship between age and breeding success and brood size.

To better determine if this quadratic effect was caused by a monotopic increase followed with a decreasing slope or a peak at intermediate age followed by a decline, we performed the analyses before and after the peak of breeding success, situated at 4 years of age for breeding success and 6 for brood size. These analyses showed that there was a strong

increase in breeding success and brood size, both for males, females and total of individuals during the first years of their life (Table 2, Fig.1). Relationships from the older ages varied according to sex and breeding parameter. For breeding success, the decrease

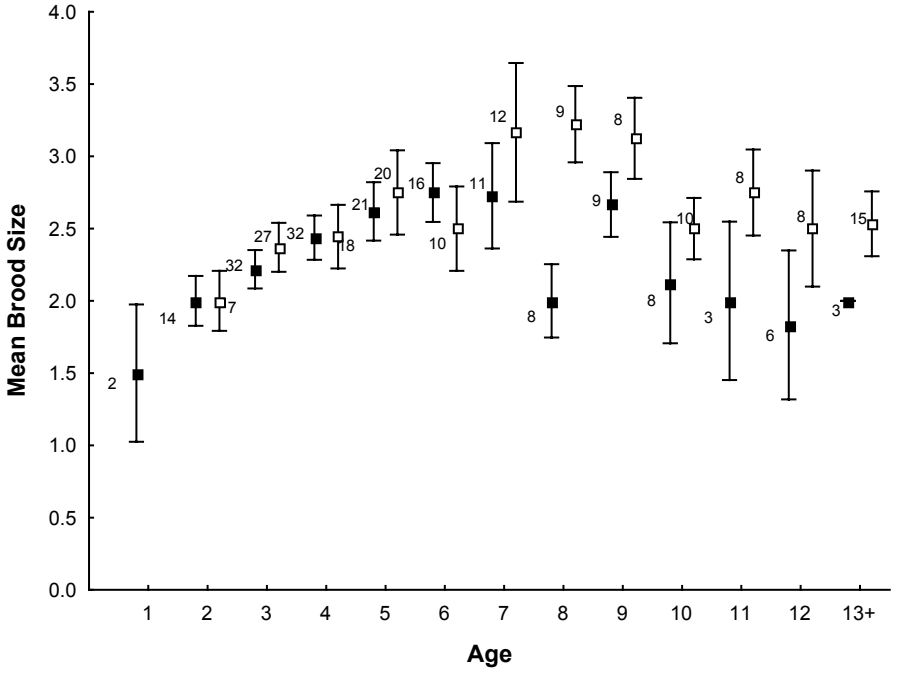

Figure 2. The distribution of mean fledging success with respect to age for white stork in the Province of Madrid. Only males (solid square) and females (shaded square) that hold nests with fledged storks are represented. All age classes include known age individuals. Age-class 13+ is a composite of individuals 13 to 17 years old. Sample sizes are indicated.

after the peak was not significant for both males and females (Table 2) However, for brood size, effects were not significant for males (Table 2) and marginally significant for females (Table 2).

An unexpected drop in the curve describing breeding success and brood size was found for males at age 8, with a recovery afterwards to the expected values (Figure 1, Figure 2)

The probability of breeding failure was analysed in relation to age by considering males and females separately after controlling for the effects of the individual identity and year, which were considered random factors. We found a quadratic effect for females, males and the total data set including non-sexed individuals (Table 3, Figure 3). Linear trends before and after the peaks of brood size were statistically significant only for males (Table 3).

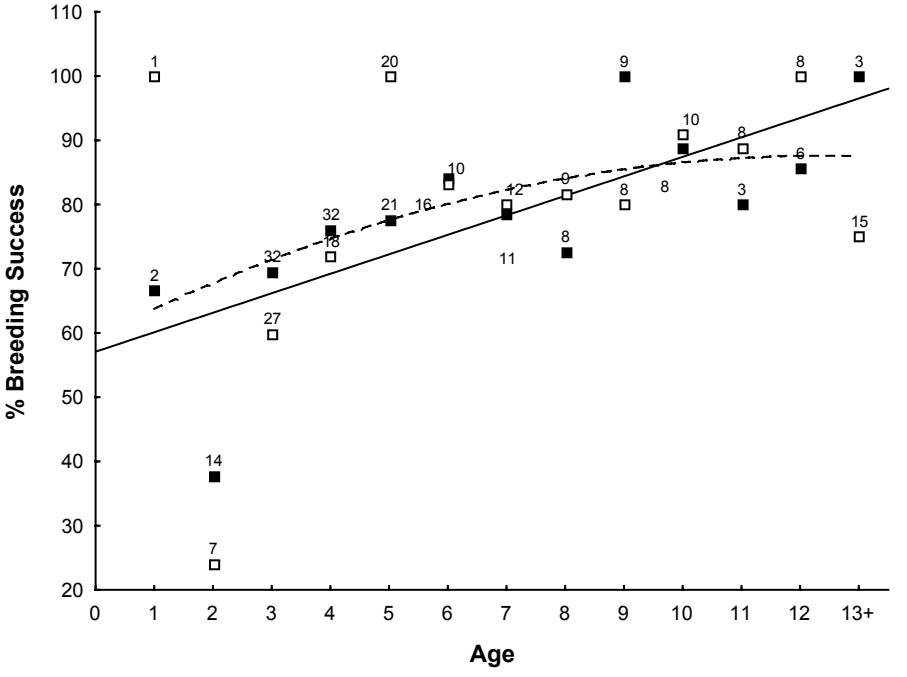

Figure 3. Percentage of male (solid square) and females (shaded square) and tendencies of female (dotted) and males (solid) that succeed on fledging young for each group of age for white stork in the Province of Madrid. All age classes include known age individuals. Number of cases is indicated.

DISCUSSION

Parents play an important role on breeding success during each breeding seasons. Individuals adopt different strategies, sometimes determined by their own life experience and others determined by environmental circumstances. Predation generally plays an important role on breeding success, although in colonial species, with a large size, such as the White Stork, this factor loses its relative influence on final reproductive output. In the population under study only 4% of the nestlings have been predated (J. Aguirre unpublished data), which indicates a low influence of predation on the survival of broods. In our population, the most important reason for chick mortality is starvation. The individual experience of parents on foraging plays therefore a key role for their breeding success. Improvements in foraging skills, breeding experience and local knowledge are generally acknowledged to be important determinants of reproductive performance

with increasing age (Forslund & Pärt, 1995). Older individuals generally have better access to high-quality resources because of age differences in dominance, timing of resource acquisition and r prior knowledge of where to find the best resources (Pärt, 2001). Old storks do not seem to have access to better breeding grounds, but they may have a better knowledge of where to find the best resources. In several experimental studies, a cost of reproduction has been shown for several bird species (reviewed by Nur (1988). Older individuals are more successful than younger ones, but they raise smaller broods due to a lower frequency of food provisioning to the nest. Breeding success at young ages is low because of the lack of experience concerning foraging skills and therefore young birds fail more often. Still, when successful, they show higher food provisioning rates, and therefore are able to raise larger broods. This occurs specially in males.

Two main mechanisms may generate age related improvement in reproductive success First, learning may enhance competence in breeding activities, like for example foraging, dominance interactions and anti-predator or parasite defence, resulting in improved quality of parental care and access to limiting resources. Secondly, the optimal level of parental effort may increase with age due to a reduction in residual reproductive value.

Furthermore, we found sex differences in the effect of senescence on breeding success. This could be due to the fact that males usually enter the breeding population at an earlier age than females, suggesting that they reach their reproductive maturity sooner. More data involving breeding pairs with both members marked would be adequate to test such hypothesis.

The drop on brood size and breeding success of males at age 8 could indicate that they reached the end of their reproductive life. High breeding effort at early age should represent high costs of reproduction and therefore earlier mortality (Milinoff *et al*., 2002). Also, older males find more difficulties to find food resources or in addition are less attractive and therefore obtain lower quality females (Laaksonen *et al*., 2001).

Females that enter too early in the reproductive population pair to less experienced males which are the ones not paired after nest defence and pair formation.

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2 Fidelidad a los lugares de nidificación y éxito reproductor en la Cigüeña Blanca *Ciconia ciconia*

Este capítulo reproduce el texto íntegro del siguiente manuscrito:

VERGARA, P., AGUIRRE J.I., FARGALLO, J.A. and DÁVILA, J.A. (*Aceptado*). Nest-site fidelity and breeding success in White Stork *Ciconia ciconia*. *Ibis* 148: 000-000.

RESUMEN

La fidelidad a los nidos es un aspecto de la estrategia vital de las aves que puede producirles beneficios en términos de eficacia biológica. Se ha estudiado la relación existente entre el éxito reproductor y la fidelidad a los nidos en la Cigüeña Blanca *Ciconia ciconia*. También se ha estudiado cómo otros factores como la edad, el sexo, el tipo de hábitat, el tamaño de colonia y la productividad en el año anterior pueden influenciar esta relación. Los resultados muestran que parejas con mayor tasa de fidelidad también tienen una menor tasa de fracaso y que el fracaso en la cría y la productividad en años anteriores influyen en la tasa de cambio durante la siguiente temporada de cría. Además, se ha encontrado una relación cuadrática entre la edad y la fidelidad a los nidos. Estos resultados sugieren que la edad es el factor más relacionado con la fidelidad al nido y por tanto, la experiencia individual puede explicar este comportamiento. El cambio de nido implica un coste reproductivo y por ello la fidelidad al lugar de nidificación puede ser considerada una estrategia para incrementar la eficacia biológica de esta especie.

Nest-site fidelity and breeding success in White Stork *Ciconia ciconia*

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ABSTRACT

Nest-site fidelity is a life history trait of birds that may produce benefits in terms of fitness. We studied the relationship between breeding success and nest fidelity in the White Stork *Ciconia ciconia*. We also studied how other factors such as age, sex, habitat, colony size and productivity in previous breeding attempts might interfere with this relationship. Our results showed that pairs with higher fidelity rates also have lower failure rates, and that breeding failure and productivity in the previous season influenced the frequency of nest change in the following season. In addition, a curvilinear relationship was found between age and nest fidelity. These results suggest that age is a major factor related to nest fidelity and, therefore, individual experience could explain this behaviour in the White Stork. Changing nest involves reproductive cost for which nest fidelity can be considered as an adaptive strategy to increase fitness.

INTRODUCTION

Nest-site fidelity is considered to be an adaptive strategy in numerous species of birds such us Procellariiformes (Bried *et al*. 2003), Anseriformes (Blums *et al*. 2002) and Ciconiiformes (Cezilly *et al*. 2000). One of the functions proposed to explain this behaviour is the strengthening of pair bonds and, therefore, mate retention (Cezilly *et al*., 2000), which could result in higher survival rates for adults (Ens *et al*., 1993; Bried *et al*., 2003) and/or contribution to the maintenance of the social status (Heg *et al*., 2003). Furthermore, the maintenance of nest sites over successive breeding seasons is related to low rates of ectoparasitism (Barclay, 1988; Rendel & Verbeek, 1996) and/or predation risks, since nest predators have a long-term memory for rewarding nest sites (Sonerud & Fjeld, 1987).

One factor that might affect nest-site fidelity is breeding success in the previous season. Pairs failing to produce, or producing fewer, offspring in a given season tend to be more likely to change nest sites before the next breeding season (Hoover, 2003). Therefore, nest-site fidelity could also be determined by age, since younger individuals tend to fail more breeding attempts than adults (Rowley, 1983; Pyle *et al*., 2001). On the other hand, older individuals produce fewer offspring due to the effect of senescence (Forslund & Pärt, 1995; Reid *et al*., 2003). Productivity, as a component of breeding success, could also modulate nest-site fidelity behaviour. Similarly, colony size has been proposed as a determinant of breeding performance in birds (Wittenberg & Hunt, 1985). Although there are costs associated with the increased size of the colony, such as high rates of parasite transmission, competition for food or for nest material (Burger, 1981; Carrascal *et al*., 1995; Brown & Brown, 1996), breeding success is, in general, higher in larger subcolonies, probably because colony size reduces predation risk (Barbosa *et al*., 1997 and references therein). Hence, colony size could mediate nest-site fidelity via breeding success. Other factors, such as sex, breeding density, habitat or nest characteristics have also been found to be related to nest-site fidelity (Switzer, 1993; Dubois *et al*., 1998; Joventin & Bried, 2001; Pyle *et al*., 2001; Johannesen *et al*., 2002; Beheler *et al*., 2003).

In the White Stork *Ciconia ciconia*, nest-site fidelity has been reported in over 80% of individuals (Barbraud *et al.,* 1999). In this species, changes of nest are more frequent in young individuals and are usually followed by mate changes, although changes in nest-site have also been observed among different breeding attempts with no particular reasons for that behaviour (Schulz, 1998). To date, there have been no studies focussed

specifically on the factors affecting nest-site fidelity in White Storks. The aim of this study is to explore the applicability, to a long-term monitored population of White Storks, of some of the factors thought to affect nest-site fidelity in birds.

First, we tested whether breeding failure or productivity in the previous breeding season affected nest-site fidelity. If breeding failure does affect nest-site fidelity, we predict higher nest-site fidelity rates in adult than in young individuals. If productivity affects nest-site fidelity, we predict a curvilinear relationship between nest-site fidelity and age. If changes in nest site are performed to increase breeding success, we also predict higher breeding failure and/or lower productivity in pairs that subsequently change nest than in pairs that did not. In addition, if colony size affects breeding success, nest-site fidelity should be correlated with this variable. Finally we analysed the relationships between nest-site fidelity and other variables, such as sex and habitat-type.

METHODS

The study was carried out in the province of Madrid (7740 km^2), central Spain. In this area the number of White Storks increased from 215 breeding pairs in 1984, (Lázaro *et al*., 1986) to 979-1013 pairs in 2001 (Aguirre & Atienza, 2002) and 1220 pairs in 2004 (J.I. Aguirre, unpublished data). White Stork breeding has been monitored in our study area since 1989. Chick ringing with metal and PVC rings has been carried out since 1980. Nests in the study area (solitary or colonial) were checked from 1989 to 2004 in order to record ringed individuals and the number of nestlings produced. For this purpose we used binoculars (8x30) and telescope (20x30-60). In total, 170 ringed individuals were identified as breeders.

Nest-site fidelity was defined as the permanence of an individual in the same nest in two consecutive breeding seasons. Breeding outcome, was considered as a binary variable where "0" was assigned to failed nests, and "1" to successful nests. Nests in which none of the eggs hatched were considered as failed nests. Productivity was defined as the number of nestlings 40 days after hatching in successful nests (range = 1–4). Only four pairs produced more than four nestlings. These cases were included in the highest productivity category (four). The size of the colony was defined as the number of nests. We considered 'urban' and 'countryside' as two different breeding habitats. The sex of ringed breeding individuals was determined by observation of copulatory behaviour at egg laying (Cramp & Simmons, 1977; Schulz, 1998). Nestlings were sexed by molecular techniques (Fridolfsson & Ellegren, 1999) using DNA

extracted from blood obtained by brachial venipuncture during banding at the age of 40 days. Molecular sexing of nestlings also allowed us to know the sex of some breeding individuals in subsequent years. Age was determined by identification of band codes of individuals marked as nestlings

Sex	Nº obs. fidelity	Nº obs. switching	Total
Males	117 (49)	33 (25)	150 (56)
Females	104 (39)	37 (31)	141 (51)
Undetermined	123 (57)	18 (15)	141 (63)
Total	344 (145)	88 (71)	432(170)

Table 1. Summary of the number of observations. The number of different individuals for each class is given in parentheses.

In order to analyse nest-site fidelity we formulated generalized linear mixed models (GLIMMIX) with binomial error distributions (Littell *et al.,* 1996) using SAS statistical software (SAS 1989-96 Institute Inc., Cary, NC, USA) in which nest-site fidelity was included as the response variable. As potential explanatory variables we considered sex, habitat type, breeding outcome of the previous year (fixed factors), colony size and age (covariates). A second model was similarly performed by using the productivity of the previous year as a covariate, instead of breeding outcome of the previous year.

To improve the function explaining the relationship between age and nest-site fidelity, we estimated the proportion of individuals that did not change nest in each age-class. Age classes showed different sample sizes, for which the contribution of each age class to the models was unequal. The analysis was adjusted by weighting the values by the number of individuals in each age class.

In order to know whether breeding outcome was associated with nest-site fidelity, we constructed generalised linear mixed models (GLIMMIX) with a binomial error distribution with breeding outcome as the response variable. As potential explanatory variables we used nest-site fidelity, sex, habitat type (fixed factors) and colony size (covariate). The bird's age, and the squared term of age, were included in the model to test the effect of senescence. We constructed general mixed models (GLMM) with productivity as the response variable. Nest-site fidelity, sex, habitat type (fixed factors) and colony size (covariate) where again included

in the model. Age and its squared term were again included in the model. As productivity did not present a normal distribution (K-S, d = 0.21, $P < 0.01$), residuals from all GLMM were checked. All the residuals were distributed normally (K-S, all $d < 0.08$ and all $P > 0.05$).

In many cases the same individual was recorded in two or more years, for which we included individual (ring number) as a random factor in all models to avoid pseudo-replication. As breeding performance is expected to vary among years, we also considered breeding year as a random factor. As some of the explanatory variables could covary, we fitted their effects to the observed data following backward and forward stepwise procedures, testing the significance of each variable one by one, and removing or adding respectively the variables that resulted in the largest increase of model fit. The result is the minimum adequate model (MAM) for explaining the variance of the response variable, where only significant explanatory variables and two-term interactions were retained. We used the Akaike's Information Criterion (*AIC*; Akaike, 1973) to determine which model best fitted the data. All the analyses were two tailed. Two-year old individuals were excluded from the analyses due to the low sample size (n=2). In order to balance the data we regarded the last age class as 14 years or older.

RESULTS

Nest-site fidelity was observed in 344 cases (79.6%) out of 432 records (Table 1). Nest-site fidelity was explained significantly by the breeding outcome of previous years and age (GLIMMIX, Breeding outcome: $F_{1,132}$=18.51, P<0.0001, *estimate*= -1.5275; age: $F_{1,132}$=14.80, P=0.0002, *estimate*=0.2843; model: *Scale Deviance*=216.93, n=245). Older individuals producing one nestling at least during the previous year tended to change the nest less often. Sex showed a marginally significant effect on nest fidelity (GLIMMIX, $F_{1,132}$=3.02, P=0.086, *estimate*=-0.6127) with females tending to change more than males. The Akaike's Information Criterion showed that the model excluding sex as an explanatory variable fitted the data better (*AIC*=1219.4) than the model including it (*AIC*=1231.4). The remaining variables were not significantly correlated with nest-site fidelity (all P >0.1). Age and breeding outcome of the previous year were intercorrelated (GLIMMIX, $F_{1,152}$=15.96, P=0.0001, *estimate*=0.2874). In order to disentangle the effect of both variables we analysed the variation in nest-site fidelity within each age-class. Breeding outcome of the previous year had a significant effect at four years of age (GLIMMIX, $F_{1,44}$=8.43,P=0.0057, *estimate*=-1.8746), seven years of age (GLIMMIX, $F_{1,16}$=6.06, P=0.02, *estimate*=-4.0833) and nine years of age (GLIMMIX, $F_{1,9}$=4.81, P=0.05,

estimate=-4.3413), but not in the remaining age-classes (all $P > 0.1$).

When we replaced breeding outcome by productivity of the previous year in the model, productivity of previous year had a significant effect on nest-site fidelity only through the interaction with sex (GLIMMIX, productivity*sex $F_{2,84}$=3.50, P=0.03, *estimate*=-0.8452). More productive, and male, individuals tended to change the nest less frequently. Sex showed a marginally significant effect on nest fidelity (P=0.16), females tending to change more than males. According to Akaike's criterion the model including sex fitted the data better (*AIC*=1310) than that excluding sex (*AIC*=1335.5). Thus we accepted the model including sex as the final model (GLIMMIX, productivity*sex $F_{2,84}$=3.69, P=0.029, *estimate*=-0.8452; age $F_{1,84}$=19.75, P<0.0001, *estimate*=0.8670; sex $F_{1,84}$=2.01, P=0.16, *estimate*=3.0018; model: *Scale Deviance*=151.97, n=181).

Age and productivity of the previous year were intercorrelated (GLMM, $F_{1,98}$=6.31, P=0.01, *estimate*=0.2915). In order to disentangle the independent effects of these variables we analysed the variation in nest-site fidelity due to productivity of the previous year within each age-class (similarly to the analysis with breeding outcome of the previous year). Productivity of the previous year was not significant in any age class (all P >0.2).

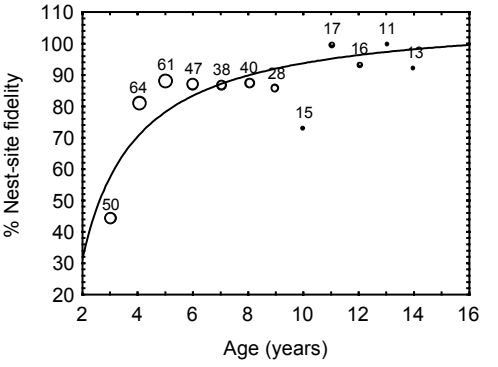

Figure 1. Correlation between age of breeders in years and the percentage of cases with nest-site fidelity, weighted by the number of observations for each age-class and adjusted according to the hyperbolic function; y= (109,31)+(-155,74)/x. Number of cases is representing with different size in symbols (least to major, 10-15, 16-20, 21-30, 31-40, 41-50 and >51.).hyperbolic function (R^2=0.81, Fig. 1) than by a linear (R^2=0.60) or quadratic (R^2=0.73) function.

The proportion of individuals not changing the nest was positively correlated with age ($F_{1,398}$=235.28, P<0.0001). This relationship was explained better by a

Controlling for age and the squared term of age, breeding outcome was significantly affected by nest-site fidelity (GLIMMIX, $F_{1,144}$=8.55, P=0.004, *estimate*=1.067; model: *Scale Deviance*=237.9, n=283). Breeding outcome was significantly lower in individuals that changed their nest sites (0.68±0.04) than in those that retained the nest (0.86±0.02). The remaining variables were not significantly correlated with breeding outcome (all P>0.5). The productivity (controlling for age and the squared term of age) was not affected by nest-site fidelity (P=0.3), but was correlated with the interaction between sex and age (GLMM, $F_{1,155}$=4.02, P=0.046, *estimate*=0.038; n=271). Females and older individuals were more productive. No other

variable correlated significantly with productivity (all $P>0.1$).

To detect whether birds that changed nest following breeding failure performed better the next year that birds that had also failed, but re-used the same nest site, we repeated the last two models, but used only cases in which birds failed to produce nestlings during the previous year. Considering only the individuals that failed in the breeding attempt of the previous year, we found that productivity was not affected by nest-site fidelity (GLMM, P=0.9). However, individuals that changed nest showed a significantly poorer breeding outcome than individuals that retained the nest in the next breeding season (0.64±0.06 and 0.96±0.06 respectively (GLIMMIX, F1,56=6.75, P=0.011, estimate=-2.8214; model: Scale Deviance=58.04, n=70).

DISCUSSION

As in other bird species, success or failure, number of nestlings produced during the previous breeding season, and age of breeders, were correlated with nest-site fidelity (Rowley, 1983; Johannesen *et al.*, 2002; Hoover, 2003). This also seems to be the case for the White Stork in our study population, where younger breeders showed lower nest-site fidelity than adults, statistically corroborating the suggestions made by Schulz (1998).

Variation in nest-site fidelity was explained by age and breeding outcome (success vs. failure) during the previous year. Breeding outcome during the previous year affected nest-site fidelity in some age-classes but not in others, for which age seems to be the main influence on an individual's decision to change nest-site. In general, storks tended to change the nest less often as their age increased. This indicates that experience plays an important role in this behaviour. It is known that young individuals occupy poorer nest sites in marginal areas and are more prone to fail in breeding. This may account for the lower rates of nest-site fidelity observed in younger individuals. As with other aspects of stork biology, such as food provisioning (Medina *et al.,* 1996), an individual's age determines its experience and therefore its use of resources. Nest-site fidelity can be influenced by an individual's experience, which allows birds better to defend their nests from other less experienced individuals (Prieto, 2002). Middle-aged individuals are able to raise their brood successfully, and have the experience necessary to maintain their nest through successive breeding seasons. Nest-site fidelity was also correlated with the productivity of the previous year, although its significance and fitness to models was lower than that observed in models with the breeding outcome of the previous year fitted as a fixed factor. However the expected decrease in nest-site fidelity in older age-classes was not observed. Older individuals

have very low rates of breeding failure despite their lower overall productivity (J.I. Aguirre pers. obs.). The significant interaction between sex and productivity of the previous year in relation to nest-site fidelity, must be analysed in future studies, because too few data have been gathered to conclude anything from our study. The significant interaction observed between age and sex in relation to productivity, could indicate than the sexes enter the reproductive population at different ages (Prieto, 2002), and this behaviour affects nest-site fidelity. This significant interaction might explain the weak differences in nest-site fidelity between sexes and the real effect of the previous year's productivity on nest-changes. Younger individuals tend to arrive later in the season (Barbraud & Barbraud, 1999) and have to choose among the available nests, finding in many cases that the nest they occupied during the previous breeding season is being defended by another individual. Males tend to arrive before females to the breeding grounds (Barbraud & Barbraud, 1999), a fact which could explain the weak sex differences in nest-site fidelity. However, this requires further research.

Nest predation could influence breeding success, since it is the main reason for nestling mortality (Martín, 1993, 1995). However, the rarity of predation observed (less than 4% of the nests during the study period, J.I. Aguirre pers. obs.), indicates that this has little effect on breeding success in our population, and therefore on nest-site fidelity. The presence of ectoparasites is known to be another factor that can promote nest changes between breeding seasons (Barclay, 1988; Rendel & Verbeek, 1996). Nest parasites were not recorded in our study, but ectoparasites at the nest site have only been observed sporadically in our population (J.I. Aguirre pers. obs.). Changing nest could affect ectoparasite load when the old nest is not occupied for a breeding season, as this may break the parasite cycle. However, few nests in our colonies (less than 5%) are unoccupied in any breeding season, and so the abundance of ectoparasites is not expected to explain significant variation in nest-site fidelity in our population.

In relation to habitat, most of the White Stork's colonies in the province of Madrid are near rubbish dumps, where storks can find enough food for the entire breeding season (Aguirre & Atienza, 2002). As access to resources is similar for all individuals in the colony, we do not expect differences in breeding success to arise from a lack of food. According to our results, coloniality and the size of colony influence neither nest switching rate nor breeding success. For this reason, although the probability of pair switching or nest improvement might increase (Prieto, 2002), these factors have not influenced the aspects studied here. The low predation risk on this population (mentioned above), makes breeding success immune to the effects of colony size, which therefore has no effect on nest fidelity. We found no relationship between habitat and nest-site fidelity because

even though the situation of the nests might be different, feeding habitats are similar for both.

Finally, our results show that individuals changing their nest through consecutive breeding seasons have higher breeding failure, even though there were no differences in the number of nestlings produced between pairs remaining at the same nests and pairs changing their nests. These results also occur in storks that failed in breeding during the previous year. This illustrates how costly nest-changes are. In the White Stork, nest changes are usually associated with divorces (Schulz, 1998). Mate changes in long-lived iteroparous birds convey costs that have probably arisen from an initial inefficiency of reproducing with a new mate, a reduced reproductive success due to waiting for a new mate, and/or higher rates of mortality or loss of social status (Ens *et al*., 1996; Heg *et al.,* 2003). Our findings support this idea. Individuals suffer from loss of fitness caused by pair change, which can be reflected in a lower breeding success. In the same way, previous experience with the same partner raises breeding success (Pyle *et al*., 2001). Even though these aspects have not been tested in this study, (although we have identified 19 pairs in which both individuals are of known age, this is insufficient to draw conclusions), they should be addressed in future studies to determine (1) the degrees of nest-site, and pair, fidelity for this species, (2) the fitness costs in cases of divorce and mate fidelity, and (3) whether these costs are equal for both sexes since many studies disentangle the effects of males and females on productivity (Goodburn, 1991; Espie *et al*., 2004). We could also determine the real function of nest-changes, rather than just the causes (breeding failure etc.). Monitoring and determining other causes for nest switching such us inter-pair interactions or nest improvement (Prieto, 2002) is a very challenging task. If stronger individuals make nest-sites unable to retain or nest improvement implies nest switching, previous year breeding performance has very little to do.

We conclude from this study that variation in nest-site fidelity in the White Stork represents an adaptive strategy to increase fitness and therefore can be considered as a defining life-history trait in this species.

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3 El orden de eclosión influye en la eficacia biológica de la Cigüeña Blanca *Ciconia ciconia*

Este capítulo reproduce el texto íntegro del siguiente manuscrito:

AGUIRRE, J.I. & VERGARA, P. **(*En revisión)***. Nestling hatching order influences subsequent fitness in the White Stork *Ciconia ciconia. Evolutionary Ecology Research.*.

RESUMEN

En aves la condición de los individuos en el nido influye en su eficacia biológica futura. En este capítulo se determina si el orden de eclosión del la Cigüeña Blanca *Ciconia ciconia*, influye en la futura eficacia biológica y qué otros factores potenciales como la edad, el sexo, y la fecha de llegada a los lugares de reproducción pueden intervenir en esta relación. Se ha controlado anualmente a una población de Cigüeñas en la Comunidad de Madrid (centro de España) entre 1999 y 2004, anotando el orden de eclosión de los pollos y su peso al nacer y al ser anillados con unos 40 días de edad. El peso no es significativamente distinto entre hermanos en la eclosión pero las diferencias se pusieron de manifiesto durante el periodo en el nido. Los primeros pollos eran más pesados que el resto de sus hermanos; sin embargo, éstos eran menos exitosos como reproductores y produjeron menos pollos que los últimos. Además los pollos más jóvenes de cada nido volvieron antes a los lugares de cría después de su migración que sus hermanos mayores. Los resultados muestran que el orden de eclosión es el principal factor relacionado con la eficacia biológica y por lo tanto, las decisiones individuales inmediatamente después de salir del nido pueden determinar la eficacia biológica futura de un individuo. La migración implica una necesidad energética y es por ello que las decisiones individuales pueden considerarse una estrategia adaptativa para aumentar la eficacia biológica. Esta es la primera evidencia de que hermanos con peor condición física al abandonar el nido superen en temporadas sucesivas a sus hermanos mayores y tenga mayor éxito reproductor que estos como adultos.

Nestling hatching order influences subsequent fitness in the white stork *Ciconia ciconia*

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ABSTRACT

In birds is accepted that conditions as a nestling influences future fitness. We assessed whether hatching order of White Stork Ciconia ciconia (Linnaeus) influences subsequent fitness and how other potential factors such as age, sex, and date of arrival to the breeding grounds may interfere this relationship. A population of white storks nesting in the province of Madrid (central Spain) was monitored annually between 1999 and 2004. Hatching order and weight at hatchings and nestlings were recorded. Weight did not differ among siblings at hatching but differences revealed throughout nestling period. First hatched nestling were heavier than the rest of the brood during the nestling period. However, first hatched siblings were less successful and produced fewer chicks than the rest of the brood as breeders. In addition, younger members of the brood returned earlier to the breeding grounds than their older siblings. Our results showed that hatching order is a main factor related to fitness and therefore, individual decisions after fledging might determine future fitness. Migration involves an energetic stress for which individual decisions can be considered as an adaptive strategy to increase fitness. This is the first evidence of siblings with poorer condition as nestlings accomplishing higher breeding success as adults.

INTRODUCTION

In birds, it is generally accepted that body condition reached at fledgling influences future survival probabilities (Weatherhead & Dufour, 2000; Cam *et al*., 2003; Brown & Roth, 2004). Sibling competition is one of the most powerful forces to explain intra-brood variation in condition and survival. Parents, especially mothers, may manipulate future sibling competition and thereby survival as early as during embryonic development, by differential allocation of resources to eggs according to the laying sequence and/or sex (Slagsvold *et al*., 1984; Weatherhead, 1985, Bednarz & Hayden, 1991; Gowaty, 1995; Eising *et al*., 2001; Blanco *et al*., 2002, 2002b, 2003). Furthermore, after hatching, parents may show skewed food provisioning in relation to chick sex or hatching order within the brood (Lessells, 2002; Ploger & Medieros, 2004) or even show direct infanticide (Tortosa & Redondo, 1992; Zielinski, 2002). Also, in species with hatching asynchrony, older siblings often out compete their younger for food and, as a result, they grow more quickly (Wiebe & Bortolotti, 1994). Nevertheless, younger siblings also may have adaptive growth strategies allowing them to maintain the pace with their older siblings (Nilsson & Svensson, 1996).

In many bird species nestling body mass is also a fitness related trait (Krebs, 1999; Clotfelter *et al*., 2000), and due to the fact that hatching order influences nestling body condition in several birds species (Wiebe & Bortolotti, 1994; Kalmbach & Becker, 2005), it has an effect on fitness (Cam *et al.,* 2003). Since brood hierarchy continues after abandoning the nest (Velando, 2000) older siblings will perform better in their first years of reproductive life (Thomas *et al.,* 1999).

In addition, the highest mortality rates in birds are produced during the first year of life (Schmutz & Fyfe, 1987; Keedwell, 2003; Roth *et al*. 2005). This period is particularly critical in migratory species as the energetic stress of the migratory journey entails an additional cause of mortality (Johnson, 2005). First hatched chicks will have lower mortality rates than the rest of the brood along the migratory journey (Cam *et al.*, 2003) due to their better nestling body condition as. First hatched siblings have, therefore, better condition both as nestlings and as adults, and in consequence, higher fitness is expected (Thomas *et al.*, 1999; Cam *et al.*, 2003).

Migration is an adaptive strategy evolved to adequate food demands of individuals to temporal food resources (Pérez-Tris & Tellería, 2002). Thus, in an evolutionary context, it is expected that changes in the temporality of food availability involve

changes in migratory habits of individuals. This episode is occurring in the Iberian population of White Stork *Ciconia ciconia* (Martínez, 1994 & Vergara *et al*., 2004). White storks have been traditionally considered a transaharian migratory bird species and up to the 1980's, the only wintering strategy was migration southwards to the Sahel area (Bernis, 1959; Cramp & Simmons, 1977; Del Hoyo, 1992). Nests are occupied from February to May in South European populations (Schulz, 1998). In Spain, individual arrival date to the breeding areas takes place between November and April, but the majority of arrivals occur in January, were older birds appear earlier than younger ones (Bernis, 1959). However, since the 1980's, some individuals have been identified in November and December in their breeding areas (Prieto, 2002), and wintering in the Iberian Peninsula has been confirmed by several authors (Marchamalo, 1994, Sánchez *et al.*, 1994). In the last two decades, this phenomenon has increased in many Spanish regions, and important wintering concentrations have been detected in Spain (Tortosa *et al.*, 1995; Vergara *et al.*, 2004), as a consequence of rapid growth and extension of rubbish dumps. This produces that feedingresources are available along the annual cycle (Martínez, 1994; Tortosa *et al*., 1995). Hence, the feeding and migratory habits of this and other species started changing over a short time interval (Tortosa *et al*., 1995, 2002; Blanco, 1996) The aim of this paper is to study if there are differences in nestling body condition related to hatching order and how they might affect breeding success as adults.

We predict that hatching body mass is not influenced by hatching order but first hatched chicks may use their competitive advantages over their younger sibling throughout the nestling period as showed in other species (Wiebe & Bortolotti, 1994).

In addition, we tested if first hatched chicks out compete their younger siblings and obtain more breeding success and bigger brood sizes during their first years of reproductive life (Thomas *et al*., 1999). We also studied if first hatched chicks are in better condition at fledging (Spear & Nur, 1994) and this fact generates an early return to their breeding grounds and occupation of their nests. Early arrival has been related with higher breeding success in migratory birds (Hotker, 2002).

METHODS

Study species

White stork is an asynchronous species with 6 hours to 2.5 days difference at hatching among chicks (Schulz, 1998). Clutch size and brood size are dependent of individuals' age (Schulz, 1998), and in the study area (see

later) these values are 3.6 (Schulz, 1998) and 2.5 (Aguirre & Atienza, 2002) respectively. Juvenile dispersion was less than 100 km between place of births and breeding areas (Tortosa *et al*., 1995b; Schulz, 1998).

Study area and general procedures

A population of white storks nesting in the province of Madrid (central Spain) was monitored annually between 1999 and 2004. During this period, a total number of 894 nestlings were marked using metal and PVC rings. We were able to assign hatching order by marking the chicks with innocuous ink and placing metallic rings around 20 days of age. In order to control for the effect of brood size on hatching order, we categorized hatching order by assigning 1 to the first hatched chick, 2 to the medium hatched chick(s) and 3 to the last hatched chick. 330 nestlings were sexed by molecular procedures (Fridolfsson & Ellegren, 1999) using DNA extracted from blood obtained by brachial vein puncture during PVC banding at an age of 40-50 days. 104 of these nestlings were observed copulating in subsequent breeding seasons. We used these data to corroborate DNA sexing. We were able to determine weight of 45 hatchlings (mean 79.05 ±1.69 SD) at hatching day, and 330 nestlings of known hatching order, sex and days of age along the nestling period (range 19-63 days) using an electronic balance (accuracy 5 g).

The study was carried out in the white stork population of Madrid province (see Aguirre & Atienza, 2002). From 1999 to 2004 we surveyed 1100 breeding pairs for mark recording. Ninety-four storks (52 males and 42 females) of 165 breeding events were recorded. Marked breeding individuals were between 1 and 5 years old. However, the only one-year old individual was excluded from the analyses and in order to balance the data we grouped the four and five year individuals in one class. Then we obtained a total of 3 age classes. We monitored nests with banded breeders by several visits. Breeding outcome was considered as a binary variable where "0" was assigned to failed nests, and "1" to successful nests. Productivity was defined as the number of nestlings 40 days after hatching in successful nests (range = 1-4). Only one pair produced more than four nestlings. This case was included in the highest productivity category (four).

Between January and June in 2003 and 2004 we monitored all marked individuals at the colonies, in order to detect their arrival date to the breeding areas. We located a total number of 135 individuals (79 males and 56 females) of known age (range= 2-5). In 97 of these, breeding success was monitored. Arrival date was defined as the day of the first observation, considering "1" of January as value"1".

Statistical Analyses

In order to test differences in hatchling mass between hatching order, we constructed a general linear mixed model (GLMM) using SAS statistical software (SAS 1989-96 Institute Inc., Cary, NC, USA). As explanatory variables we included sex, hatching order (fixed factors) and laying date (covariate). Nest and breeding year were included as random factors.

In order to know whether nestling weight was explained by hatching order, we constructed a GLMM in which nestling weight (response variable); sex and hatching order (fixed factors) were included. Also we included days of age of nestlings as covariate to controlling for this variable. We did not include laying date in the last model because this variable was highly correlated with days of age of nestlings (GLMM $F_{1,157}$= 320.97, P < 0.0001, estimate =-0.7554). Nest and breeding year were included as random factors.

To test the effects of hatching order on arrival date at the colony, breeding outcome and productivity as an adult, we used mixed models. When the response variable was binomial (breeding outcome) we applied a generalized linear mixed model GLIMMIX with binomial error distribution (Littell et al. 1996), and when the response variable was continuous (productivity and arrival date) we applied GLMMs. In these models we included hatching order, sex (fixed factors) and age of breeding adults (covariate). We also included the interaction between sex and hatching order in all models.

When variables did not present a normal distribution (SW-W=0.95, P < 0.05), we checked residuals from models. In all cases, except in arrival date, residuals showed a normal distribution (Lillefors P > 0.05), for which, the use of GLMMs was suitable. The inverse transformation was used for arrival date (which had a normal distribution), although for descriptive purposes, untransformed values are presented in the figures.

We conducted analyses at the population level. They included all data across individual birds within years independently of the number of years that particular individuals were recorded as breeders. In models in which we included data of two or more years from each individual breeder, we considered individual identity as a random factor to avoid pseudoreplication (Hurlbert, 1984). As breeding performance is expected to vary among years, we also considered breeding year as a random factor. Some of the explanatory variables could covariate, thus we fitted their effects to the observed data following backward and forward stepwise procedure, testing the significance of each

variable one by one, and removing or adding respectively only the variable that resulted in the largest increase in model fit. The result is the minimum adequate model (MAM) for explaining the variance of the response variable, where only significant explanatory variables and two-term interaction were retained. All tests are two-tailed.

RESULTS

Hatchling weight was not significantly explained by sex, hatching order or laying date (all $P>0.4$). On the other hand, nestling weight was significantly explained by hatching order (GLMM: hatching order $F_{2,155}= 3.62$, $P = 0.029$). Also, sex (males being heavier than females) and days of age (positively correlated) explained differences in nestling weight. The interaction between hatching order and sex was not significatively correlated with nestling weight ($P > 0.1$) The first hatched chick was the heaviest (mean 3397.7 ± 53.82 SE4), with only little difference between middle (mean 3314.28 ± 54.60 SE) and last hatched chicks (mean 3270.08 ± 55.67 SE). Post-hoc comparisons showed significative differences between the first hatched and the last hatched ($P = 0.0095$), and more close to the significantly between first and middle hatched ($P = 0.072$). However we did not find differences between middle hatched and lasted hatched ($P = 0.353$). For this reason we joined the middle and last hatched chick into one group (hereafter rest of brood). When using this new hatching order (first chick versus rest of the brood), hatching order did indeed explain significantly nestling weight (GLMM: hatching order $F_{1,155}= 6.28$, $P = 0.013$, *estimate* =-102.75) and the other explanatory variables remained significant in the model (GLMM sex: $F_{1,155}= 7.34$, $P = 0.0075$; days of age: $F_{1,155}= 152.45$, $P < 0.0001$, *estimate* =39.4844; n=330). According to these results we established this model as the final one, and in the successive models we used the new hatching order assignment.

The mean age of marked breeding individuals was not significantly different between hatching orders (ANOVA $F_{2,163} = 0.32$, $P = 0.726$). Also, between first hatched and the rest of the brood (ANOVA $F1_{1,164} = 0.47$, $P = 0.493$).

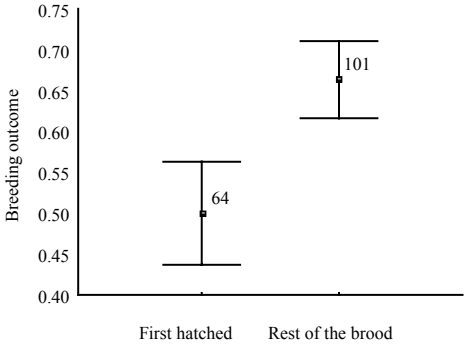

Figure 1. Differences in breeding outcome between first hatched chick and the rest of the brood. Breeding outcome: 1 = successful nests and 0 = failed nest. Point and bars represent mean and 1 SE. Sample sizes are indicated.

Controlling for the age of breeding individuals (older individuals are more

successful than younger), first hatched chicks were less successful as breeders than the rest of chicks (GLIMMIX age: $F_{1,67}$= 21.28, P < 0.0001, estimate =1.2907; hatching order: $F_{1,67}$= 5.93, P = 0.0176, *estimate* =0.9526; model: *Scale Deviance*=168.71, n=165; Fig 1). Sex and the interaction between sex and hatching order were not significantly correlated with breeding outcome (all P >0.1).

Breeding performance, as reflected in productivity, was affected by age and hatching order of the breeding adult. First hatched chicks showed worse breeding performance later in life, these individuals raised less chicks per breeding attempt than later hatched individuals. (GLMM: age: $F_{1,27}$= 7.45, P = 0.011, estimate =0.3987; hatching order: $F_{1,27}$= 4.23, P = 0.0494, *estimate* =0.3807; n=99; Fig 2). Sex and the interaction between sex and hatching order were not significantly correlated with productivity (all P >0.1).

Controlling for age of breeding individuals (younger individuals arrive later than older individuals), first hatched chicks arrive later than the rest of chicks (GLMM: age: $F_{1,52}$= 55.80, P < 0.0001, estimate =0.0070; hatching order: $F_{1,52}$= 8.39, P = 0.0055; n=231; Fig 3). Sex and the interaction between sex and hatching order were not significantly correlated with arrival date (all P >0.1). Also, when we only included individuals with known breeding outcome in the model, first hatched chicks arrive later than the rest of chicks (GLMM: age: $F_{1,34}$= 15.73, P = 0.0004, estimate =0.0041; hatching order: $F_{1,34}$= 4.16, P = 0.0492, *estimate* =0.0039; n=132)

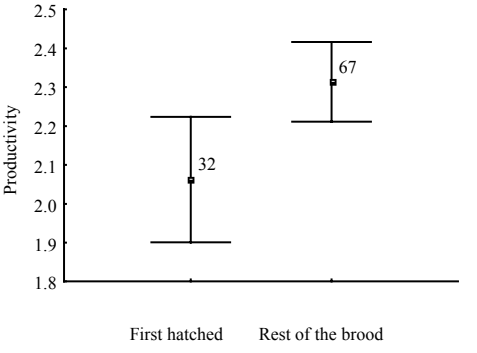

Figure 2. Differences in productivity (number of nestlings produced) between first hatched chick and the rest of the brood. Point and bars represent mean and 1 SE. Sample sizes are indicated.

DISCUSSION

As found in other bird species (Clotfelter *et al.,* 2000; Massemin *et al*., 2002), white stork hatchlings did not show differences in body mass according to hatching order. However, from the second week, first hatched chicks reached better body mass than last hatched chicks, probably because of their larger size conferring them competitive advantages in sibling competition (Wiebe & Bortolotti, 1994). This result suggests that hatching first provided these nestlings with a growth advantage. In several species, body mass at fledgling was related to subsequent

survival (Krebs, 1999; Clotfelter *et al*., 2000), and thereby, nestling body mass is a fitness related trait. If first hatched chicks are favoured by at

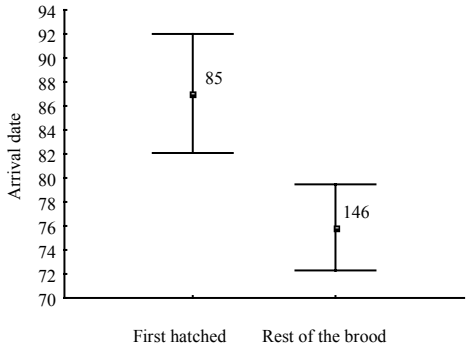

Figure 3. Differences in arrival date between first hatched chick and the rest of the brood. Arrival date: 1=1 January. Point and bars represent mean and 1 SE. Sample sizes are indicated.

least a larger body mass over their younger siblings, they are expected to survive better. However, an individuals' fitness is reflected by its lifetime reproductive success, and therefore it is interesting to study long-term effects of nestling traits on reproductive success later in life. Some studies found long term effects of nestling body mass. We found that first hatched white stork nestlings performed worse in their firsts breeding attempt. To our knowledge this is the first evidence in which siblings with the poorer condition as nestlings, have the higher breeding success as adults. The apparent benefit of first hatched nestlings of a larger body mass was not reflected in breeding success in the subsequent years. First hatched nestlings failed more often in their early breeding attempts, and when achieved successful breeding, produced less offspring. However, although first hatched nestlings raised fewer chicks per breeding attempt in their first years of life, it is possible that they live longer and therefore, balance their life fitness at the end. This requires further research, with complete life histories for each hatching order category.

A possible explanation for differences in breeding success between hatching order categories may be found in different migratory strategies between young birds. Once fledglings abandon the nest, parental care decreases and fledglings must develop individual strategies to guarantee their own survival and future fitness. One of the strategies to consider is the migratory journey. This journey represents one of the biggest energetic stresses a bird must undergo throughout its life (Schaub & Jenni, 2001). Later hatched chicks have a higher probability to fail a migratory journey, probably because of their lower body mass (Michard *et al*., 1997). Nowadays, rubbish dumps and the recent increasing population of Crayfish *Procambarus clarkii* mainly in rice fields in the Iberian Peninsula represent valuable sources of food for white storks available during winter (Martínez, 1994; Sánchez *et al*., 1995; Blanco, 1996). Due to this recent change in food availability, later chicks might benefit from their *a priori* disadvantage. Bad

condition at the beginning of their life could save the last chicks of the brood from a high probability of failure on a migratory journey. Some kind of counter selection based on physical condition could act, so the worst individuals obtain bigger adaptive advantages. This particular point indicates plasticity of selection over new man-induced circumstances like new food resources. Therefore, we are facing a very interesting experiment at a population level to understand the evolutionary plasticity of the populations and their adaptive capacity to new circumstances.

Why first hatched chicks pass over the new resources and migrate to their traditional wintering sites? Non-migratory behaviour is produced rather than from the abundance of food, from the lower capability of weaker individuals to face the migratory journey. Therefore, if new environmental food conditions favour survival of not migrating young birds, this behaviour might be an adaptive strategy for later hatched chicks to shorten migration or not migrate at all. If these birds chose the same strategy in subsequent years, not migrating later hatched chicks could consistently return earlier to their breeding grounds compared to migrating first hatched chicks. However, further studies are necessary to test this interesting possibility, and to test for benefits and/or the reason of migrating of first hatched offspring.

The earlier arriving of later hatched chicks could result directly in an earlier start of breeding, which has been shown to lead to higher reproductive success (Tryjanowski *et al.*, 2004). On the other hand, earlier arriving may also minimize the time needed to search and occupy a good nest site (Tryjanowski *et al*., 2004) and thereby increase the time available to accumulate resources, obtaining a better physical condition before breeding. Parental physical condition has previously also been shown to influence breeding success in this species (Sasvari & Hegyi, 2001). This last possibility more likely explains the differences we found in breeding success between first and later hatched chicks. Early breeders are probably of better quality, but individuals arriving earlier at the colony are not necessarily. Food abundance also influences both laying date and brood size (Newton & Marquiss, 1981; Askenmo, 1982; Aparicio, 1994; Tortosa *et al*., 2003). Natural selection might benefit both early arrives and early breeders and their individual condition would be factor to chose one or another (Forstmeier, 2002). Even though altering migratory arrival has very high energetic costs (Kokko, 1999) sometimes benefit overcomes costs (Forstmeier, 2002). Also further studies are needed to test the hypotheses proposed to explain the results we found in the present study.

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4 Sexo y equilibrio entre respuesta inmune mediada por celulas T: decisiones parentales en la Cigüeña Blanca

Este capítulo reproduce el texto íntegro del siguiente manuscrito:

AGUIRRE, J.I. & BANDA, E. (*En revisión)*. Sex and trade-off between growth and t-cell mediated immunity: parental decisions in the white stork. *Canadian Journal of Zoology*.

RESUMEN

Las decisiones potenciales de los padres, pueden influenciar las futuras variables reproductivas de sus hijos. En el siguiente capítulo, se han investigado las relaciones entre la respuesta inmune mediada por células T en una especie con machos más grandes que hembras como es la Cigüeña Blanca *Ciconia ciconia* durante una temporada de campo, con parámetros como el sexo o el orden de puesta. Los resultados sugieren que la respuesta inmune decrece con el orden de puesta y con el peso del huevo. Las hembras han mostrado una respuesta inmune mejor que los machos. Estos resultados sugieren que existe un equilibrio entre la energía dedicada al crecimiento y la dedicada al desarrollo de un sistema inmune. Esta variable debe estar mediada por el efecto inhibidor de la testosterona, que implica machos con menores niveles de respuesta inmune que hembras proporcionalmente más grandes

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Sex and trade-off between growth and T-cell mediated immunity: parental decisions in the white stork

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Abstract

Potential parental decision might influence future traits of nestlings. In the present study, we investigate the relationship between T-cell mediated immune response in the sexually dimorphic White Stork Ciconia ciconia attending to traits such as sex and sibling competition during embryonic and nestling period. Immune response decreased with hatching order and egg weight. Females showed higher response than males. Our results reveal trade-offs between growth and development of immune system.

INTRODUCTION

Parents, especially mothers, may manipulate future nestling composition during embryonic development by differential allocation of resources to eggs according to the laying sequence and/or sex (Gowaty 1995; Eising et al. 2001; Blanco et al. 2002; Blanco and De La Puente 2002; Blanco et al. 2003). Furthermore, after hatching, parents may show skewed food provisioning in relation to chick sex or hatching order within the brood (Ploger and Medeiros 2004).

At nestling stage, T-cell mediated immune (CMI) response is a good estimator of the general state of health and future survival of chicks (Horak et al. 1999; Soler 1999; Tella et al. 2000). The acquisition of CMI can be partly environmental, mainly through the positive effect of nutrition (Saino et al. 1997; Fargallo et al. 2002; Jovani et al. 2005) but it also has a genetic component (Johnsen et al. 2000). CMI grows along with the rest of the body structures since white cell formation needs the same vitamins and amino acids for its formation than the rest of the body (Soler 1999). Compromise between growth and CMI usually starts at early stages of development (Muller et al. 2003). It has been found that the maintenance of the immune system is energetically and nutritionally costly (Lochmiller and Deerenberg 2000), which explains why immune response is commonly associated with fledgling body mass and condition (Alonso-Alvarez and Tella 2001; Fargallo et al. 2002; Laaksonnen et al. 2004) and indicating that immunocompetence is a food-dependent function when chicks are growing. Therefore, although parental influence have an effect on nestling CMI response, the knowledge about these questions in wild birds is still scarce.

Sex differences in CMI response have been found in the Eurasian Kestrel *Falco tinnunculus* (Fargallo et al. 2002) a sexual size dimorphic species. Two hypothesis have been proposed to explain such trait: the *competitive capacity* hypothesis which indicates that larger sex have advantages in sibling competition than smaller sex and *immunocompetence handicap* hypothesis, which indicates that males have lower CMI response due to the trade-off between the effect on the production of secondary sexual characters by testosterone and its inhibiting effect on the immune system (Folstad and Karter 1992) both could be related to parental decisions (Lessels and Krebs 1989; Ploger and Medeiros 2004).

The aim of this paper is to explore the incidence of potential parental decisions in CMI response of nestlings in a sex dimorphic species such as the White Stork while food

provisioning remains constant. Tests to determine whether if CMI is related to sex, and sibling competition during embryonic and nestling period will be accomplished. To our knowledge this is the first time that relations between immune system and weight of eggs will be tested it extends the scopes of the embryonic development hypothesis ((Ricklefs 1992; Tella et al. 1999).

METHODS

CMI response of nestlings was studied in a colony of White Storks in the Northeast area of the Province of Madrid during the breeding season of 2003. The proximity of one of the biggest rubbish dumps in the province makes possible for this birds to have a reliable and constant source of food (Donázar 1992; Tortosa et al. 2002; Tortosa et al. 2003). All the nests studied are sited in Ashes *Fraxinus angustifolia*, allowing the assessment of clutch size (number of eggs laid). Several visits to 54 different nests randomly selected within the colony were undertaken to record the exact laying date and incubation period for complete clutches. Mass of eggs was measured (to the nearest 1g.) at laying time. Egg hatching was monitored and all nestlings were assigned hatching order by marking them with innocuous ink and placing metallic rings at 20 days of age. Hatching order was scored by assigning 1 to the first hatched chick, 2 to the medium hatched chick(s) and 3 to the last hatched chick (range of clutch size 2-6 eggs). Brood size, defined as the number of nestlings 40 days after hatching was recorded at this moment. 109 individuals (48 males, 67 females) from 46 nests were sampled for CMI between 35 and 50 days of age (median 42 days). Body mass (to the nearest 5g.), bill length and tarsus of nestlings was measured at hatching and at the time of skin swelling measurement. At this age, a phytohaemagglutinin-P (PHA) injection assay (Cheng and Lamont 1988) was used to evaluate the in vivo T-cell-mediated immune response of storks. Birds were injected intradermally with 0.1 ml of 2mg/ml phytohaemagglutinin-P (Sigma) in PBS solution. We have used the simplified protocol proposed by (Smits et al. 1999), which avoids the injection of PBS in the opposite structure as a control. The advantages of eliminating the PBS injection are a decrease by half in the handling time of the birds (and thereby in stress), a reduction in the probability of errors when injecting into thin structures such as wing or foot webs, and a decrease in the coefficient of variation that is due to measurement inaccuracies (Smits et al. 1999). The thickness of each foot web area was measured with a digital calliper (to the nearest 0.01 mm) at the injection site, marked with a permanent marker, before and 24 hours after the injection. The cell-mediated immune response was estimated as logarithm of the difference in thickness of the right foot web

from the day of injection to the following (Smits et al. 1999). The same researcher took three measurements of each web on each occasion and the mean was used in subsequent analyses. Nestlings were sexed by molecular techniques (Fridolfsson and Ellegren 1999) using DNA extracted from blood obtained by brachial venipuncture during ringing. The same method has already been tested in storks by (Jovani et al. 2005).

CMI response showed a normal distribution. (SW-W=0.98, P > 0.05). In order to test differences in CMI response, we constructed a general linear mixed model (GLMM) using SAS statistical software (SAS 1989-96 Institute Inc., Cary, NC, USA). As explanatory variables we included hatching order, sex (fixed factors), clutch size, laying date, egg weight, days of incubation, number of hatchlings (covariates). We also included nestlings body mass at CMI assay to control for this variable. To evaluate if each sex responded in a similar way in relation to the above variables, we added the interactions between sex and all the previous variables to the model. Nest was included as a random factor to avoid pseudoreplication.

Some of the explanatory variables could covariate, thus we fitted their effects to the observed data following backward and forward stepwise procedure, testing the significance of each variable one by one, and removing or adding respectively only the variable that resulted in the largest increase in model fit. The result is the minimum adequate model (MAM) for explaining the variability in the response variable, where only significant explanatory variables and two-term interaction were retained. We used the Akaike's Information Criterion (*AIC*; (Akaike 1973) to determine the model that fitted the data better, when two alternative models resulted. All tests are two-tailed.

Table 1. GLMM with normal error and identity link function on CMI response. Only significant variables are presented

Variables	***Estimate (S.E.)***	***d.f.***	***F***	***P***
Egg weight	-0.00274 (0.001039)	1, 56	6.96	0.0108
Hatching order	0.1034 (0.03951)	2, 56	3.53	0.0361
Sex	0.4441 (0.2035)	1, 56	4.76	0.0333
Body mass	0.000165 (0.000051)	1, 56	7.83	0.0070
Sex*body mass	-0.00015 (0.000062)	1,56	5.73	0.0200

RESULTS

Egg weight was not significantly explained by sex, hatching order or their interaction (GLMM, all $P>0.2$). CMI response was significantly explained by egg weight, hatching order, sex, and interaction between sex and body mass (Table 1). CMI decreased with hatching order (*estimate*=0.1034) (Fig.1) and egg weight (*estimate*=-0.00274). Females showed higher CMI response than males (Fig 2). Also, heaviest females showed highest CMI-responses (*estimate*=0.000165). This trend was not observed in males (Fig.3). The rest of the variables and their interactions with sex did not affect significantly CMI (all *P*-values > 0.05)

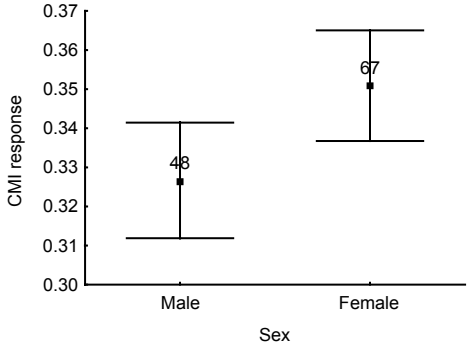

Fig. 1. Relationship between sex of nestlings and CMI response of White Stork. Note that error bars depict 95% standard error. S

In white stork, males are slightly bigger (up to 5% (Schulz 1998) than females). Immune organs of males are generally less developed than females', such differences are

DISCUSSION

It is generally assumed a trade-off between immune response and growth (Lochmiller and Deerenberg 2000; Soler et al. 2003). Thus bigger individuals are harder to produce (Torres and Drummond 1999). Different allocation of resources during embryonic development can be achieved by parents in order to manipulate future nestling composition (Magrath et al. 2003). This difference should be enough to unequal balance and sacrifice immunocompetence capacity in order to obtain larger sizes even at embryonic development. The decline in egg weight and therefore in the amount of available resources might be responsible for the observed decrease in CMI. caused by the immunosupresive effect of testosterone (Folstad and Karter 1992; Moreno et al. 2001). Our results clearly reflect such tendencies with males with lower CMI response than females at late stages of development in the nest. Such sexual differences may cause a higher prevalence of infections and parasites in males with ecological and biological consequences such as reduced reproductive success (see (Lochmiller and Deerenberg 2000) for review) or early senescence (Aguirre & Blanco unpubl. data).

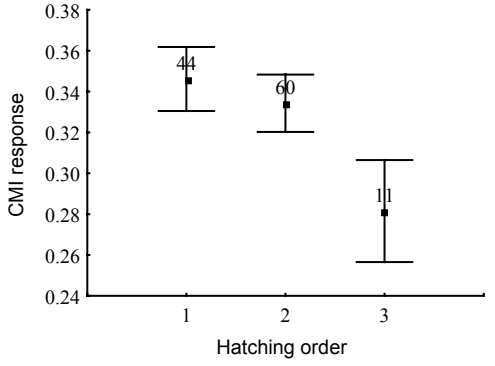

Fig. 2. Relationship between nestling category (1 first hatched chick, 2 medium hatched chick(s) and 3 last hatched chick of nestlings) and T-cell-mediated immune response of White Stork. Note that error bars depict 95% standard error. Sample size is indicated.

According to our data heavier females have higher CMI response than light males. Therefore, it is to think that the CMI inhibiting effect of testosterone could be stronger than the trade-off between growth and development of immune system. Further studies should be addressed in the future to determine at which particular state of the development such differential allocation of resources can be achieved.

Nutrition and immunocompetence are positively correlated (Lochmiller et al 1993) therefore, food availability influences immune capacity (Hoi-Leitner et al. 2001). Allocation of resources from the parents in nests with high mortality of young nestlings such as White Storks (Sasvari et al. 1999) usually favours older nestlings (Ploger and Medeiros 2004). It is generally assumed a lower body mass for last hatched chicks (Heg and Van Der Velde 2001; Saino et al. 2001). Our data reflect a lower CMI response of last hatched chicks in line with other studies (Muller et al. 2003). In addition to egg weight, the actual composition of the yolk might be of importance. Yolk testosterone levels increase over the laying sequence (Sockman and Schwabl 2000) reducing CMI response through the inhibiting factor of such hormone. Our data reflect such effect by lower CMI response of late hatched chicks.

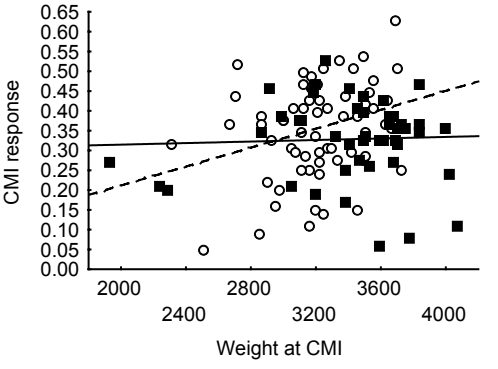

Fig. 3. Weight at CMI of male (solid square) and females (shaded circle) and linear tendencies of female (dotted) and males (solid) in relation to CMI response.

Environmental variables might not play an important role on CMI response rather than in chick survival. Temporal variability on CMI response suggested by (Jovani et al. 2005) might be caused by fluctuant food availability conditions of the particular season in which the experiment is taking place but a general relationships between body condition and CMI arise in most years. This particular case represents a good example of parental allocation of resources and hormones determines CMI response.

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